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14. ABSTRACT: : Project 1 We will determine the early molecular changes in STIC and their biological significance in developing high-grade serous carcinoma. marker selection and sample preparation will begin in the next coming months. Project 2 We will evaluate whether the presence of a STIC is associated with different clinical manifestations and/or outcome compare to those patients in whom a STIC was not identified. Molecular profiling will be initiated after quality control checking. Project 3 We will identify the early molecular changes that precede the development of STICs using gene expression analysis of morphologically normal FTE from high-risk women compared to FTE from normal control specimens and use an <i>in vitro</i> system and a mouse model to generate a molecularly defined carcinoma resembling HGSC from FTE and OSE using oncogenes expressed in ovarian carcinoma. Project 4 We plan to see if the statin drugs are effective in preventing STIC formation and suppress tumor progression in the OVGp1 mouse model that spontaneously develops STIC and neoplasms.. Project 5 With the data and cases piling up, we will be able to address the molecular and epidemiologic profile of putative precursor lesions including STIC in the fallopian tubes and ovaries from women at high-risk for ovarian cancer. Also, a pilot study will be performed to determine the most cost-effective way to prepare the tissue sections for studies related to study early tumor development in ovarian cancer. This information will be shared with science community.				
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Section I- Purpose and Scope of the Research Effort (all projects)

The purpose of this Ovarian Cancer Consortium is to test the overarching hypothesis that serous tubal intraepithelial carcinoma (STIC) is the precursor and not a metastasis of many, if not most, pelvic high-grade serous carcinomas (HGSCs) but we believe all the other proposed candidates should be investigated in order to determine if STIC is the precursor of all ovarian and pelvic HGSCs or that OSE and CICs harbor precursor lesions as well. Our objective is to then carefully characterize the morphologic, molecular genetic, immunohistochemical (IHC) and epidemiologic features of the precursor lesions(s) (Projects 1-5). If STIC is shown to be the precursor lesion, the data generated by our studies will provide the rationale for our long-term objective, which is the prevention of ovarian HGSC by surgical or medical approaches. Opportunities in the field of cancer prevention have never been greater and therefore our Consortium will undertake innovative studies aimed at providing the scientific underpinning for reducing the burden of ovarian cancer through prevention. Finally, it is important to note that clear cell, endometrioid and mucinous carcinomas are clinically important but they represent only 25 % of all ovarian carcinomas and account for 10% of deaths. In contrast, as noted above, HGSC represents 75% of all ovarian cancers and accounts for 90% of the deaths.

Accordingly, we will focus our studies exclusively on the early events associated with HGSC, as it clearly is the most important histologic subtype in terms of frequency and mortality.

The main research efforts in this Consortium are summarized in our five projects.

Project 1: Evaluate whether STICs are precursor lesions and not metastases from a primary ovarian HGSC by analyzing STICs from women with concomitant ovarian HGSCs and determining if the ovarian tumors have acquired additional molecular alterations compared to the STICs which would confirm that STICs are precursor lesions.

Project 2: Evaluate all the proposed site of origin (FTE, OSE, CICs and peritoneum) showing that the morphologic and molecular features of tubal, ovarian and primary peritoneal HGSCs are the same and in conjunction with Project 1 confirming our hypothesis that many, if not most, HGSCs originate in the fimbria and involve the ovary secondarily.

Project 3: Identify the early molecular changes that precede the development of STICs using gene expression analysis of morphologically normal FTE from high-risk women compared to FTE from normal control specimens and use an in vitro system and a mouse model to generate a molecularly defined carcinoma resembling HGSC from FTE and OSE using oncogenes expressed in ovarian carcinoma.

Project 4: Locate and characterize precursor lesions of "ovarian" cancer in a mouse model and explore the role of ovulation and changes in the microenvironment of the ovary and tube in "ovarian" carcinogenesis using human tubal xenografts in nude mice.

Project 5: Determine the molecular and epidemiologic profile of putative precursor lesions in the fallopian tubes and ovaries from women at high-risk for ovarian cancer. In addition, Project 5 will determine if these biomarkers and associated precursor lesions are modifiable by oral contraceptives (OCPs) or anti-inflammatory agents as OCPs in particular are known to prevent ovarian cancer and impact survival.

Section II, III and IV (5 projects are reported here individually)

Project 1: Evaluate whether STICs are precursor lesions and not metastases from a primary ovarian HGSC by analyzing STICs from women with concomitant ovarian HGSCs and determining if the ovarian tumors have acquired additional molecular alterations compared to the STICs which would confirm that STICs are precursor lesions.

Research site: Johns Hopkins University

Project Leader: Ie-Ming Shih

Co-investigators: Doug Levine (MSKCC), Robert J. Kurman (JHU)

Section II. Progress to Date

Task 1. Determine the clonal relationship and tumor progression pathway from STIC to invasive high-grade serous carcinoma (HGSC).

Task 1a. Case selection and sample preparation including LCM, DNA extraction (1-20 months)

Progress: We have made progress in case selection and have assessed the quantity and quality of microdissected STIC and HGSC from the same patients. As described in previous progress report, we have performed global exome sequencing on 8 HGSC and 5 normal specimens and the results appeared very good and informative. Genomic DNA was purified from 8 HGSCs and 5 normal fallopian tube specimens and enriched for coding regions (20,766 coding genes, 50Mbp) using in solution DNA capture approach. Enriched HGSC and normal genomic DNA was sequenced using massively parallel sequencing instruments (100bp paired-end). In overall, we identified 56 ~ 127 (mean 84.5) somatic non-silent mutations in each HGSC. In two patients, more than one HGSC were sequenced, allowing us to construct the phylogenetic trees for these HGSCs (figure). In these two patients, 32 and 28 non-synonymous mutations were identified as "trunk mutations", which were present in all HGSCs at different locations. Assessing whether these trunk mutations were present in the corresponding STICs will likely help elucidate the chronologic relationship between STIC and HGSC. However, the quantity of micro-dissected STICs was limited and insufficient for exome sequencing as expected. We have consulted several experts in this field to seek advice for this technical difficulty (insufficient amount of DNA in STIC). The consensus is that the problem is not our technique in extracting DNA but the nature of those STICs, being too small. As a result, we have to collect the very big STIC lesions which are rare to encounter. We agree and have consequently collaborated with pathologists from this consortium (Drs. Parkash, Shaw and Soslow) and outside this consortium. At this moment, we have fortunately made substantial progress in identifying three extremely rare cases of very big STICs. The Pathology Core is in progress in requesting those cases from our collaborators.

Task 1b. TP53 mutational analysis of all the potential precursor lesions of HGSC (8-24 months).

Progress: The task has been completed and the data have been published in J Pathol, 226:421-426, 2012. PMID:21990067. So we will not reiterate the progress here. Of note, this paper has been highly cited (more than 100 times) since publication.

Task 1c. Allelic imbalance assay by digital SNP analysis and data analysis (24-36 months)

Progress: The purpose of this task is to compare chromosomal instability among normal fallopian tube epithelium, STIC and HGSC from the same patients to elucidate the possible molecular relationship of STIC and HGSC. We have now added *CCNE1* FISH analysis in this study and have finished the first draft for publication. The final results from this study related to this task are summarized below.

In order to further characterize STIC with emphasis on molecular events involved in transition from STIC to HGSC, in this report, we studied two related markers, *CCNE1* copy number and centrosome amplification, in a series of STICs and HGSCs, many of which were concurrent and were derived from the same patients. The reason to focus on *CCNE1* is because this gene encodes cyclin E1 which binds to cyclin E1-associated kinase (CDK2) and plays an important role not only in cell cycle progression (G1 to S phase transition) but also in centrosome duplication, a strictly regulated process to maintain genetic stability(20). Overexpression of cyclin E1 can be detected in many HGSCs as well as in some STICs (14). In fact, *CCNE1* gene amplification represents one of the most common molecular genetic alterations that characterize HGSC, especially in those that develop resistance to platinum-based chemotherapy (21-24). As in many other types of solid tumors,

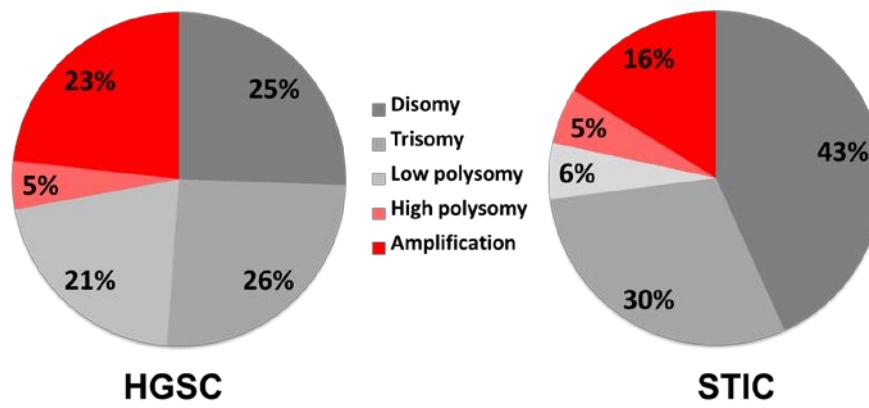
amplification and overexpression of *CCNE1* are associated with increased chromosomal instability and worse clinical outcome in HGSC patients (25-27). One of the main mechanisms how cyclin E1 overexpression contributes to aggressive clinical behavior in cancer is that abundant cyclin E1 proteins promote aberrant centrosome duplication where more than two centrosomes appear in a cell, creating chromosomal instability after cytokinesis (20, 28). Chromosomal instability, in turn, fuels the velocity of tumor evolution as it provides an expanding repertoire of tumor subpopulations to develop drug resistance and other highly malignant phenotypes under a variety of selective pressures in tumor microenvironment. Indeed, multivariate survival analysis of the Cancer Genome Atlas of ovarian cancer shows that a higher degree of chromosomal aberrations which reflecting a history of chromosomal instability was significantly associated with a worse overall survival in HGSC patients(29). The current study applies fluorescence *in situ* hybridization and immunofluorescence to analyze for *CCNE1* DNA copy number and centrosome number, respectively, on tissue sections. By comparing STICs and HGSCs, we determined whether chromosomal instability as reflected by both markers occurred early during tumor progression of HGSC and the temporal sequence for tumor cells to acquire these aberrations. A higher level of chromosomal instability in HGSC as compared to STIC will also help support the tubal origin of HGSC.

CCNE1 copy number was measured in a total of 37 STICs and 43 HGSCs using a two-color fluorescence *in situ* hybridization (FISH) assay. Among the 43 HGSC patients, 19 have concurrent STICs available for analysis and 11 of them have second STICs. The copy number was classified into five categories- gain/amplification, high polysomy, low polysomy, trisomy and disomy. In this study, *CCNE1* gain/amplification and high polysomy were considered as FISH positive. We found that 8 (22%) of 37 STICs were *CCNE1* FISH positive, of which 6 had amplification and 2 had high polysomy (Fig. 1 and Table 1). Interestingly, one out of 5 STICs not associated with HGSC showed *CCNE1* high

Table 1. Correlation between *CCNE1* copy number by FISH in 19 primary HGSCs and concurrent STICs

Copy number category	HGSC n (%)	1 st STIC n (%)	2 nd STIC n (%)
FISH negative	14 (74)	14 (74)	9 (81)
Disomy	5 (26)	6 (32)	4 (36)
Trisomy	5 (26)	7 (37)	4 (36)
Low polysomy	4 (21)	1 (50)	1 (9)
FISH Positive	5 (26)	5 (26)	2 (19)
High polysomy	1 (5)	1 (5)	
Amplification	4 (21)	4 (21)	2 (19)
Total	19 (100)	19 (100)	11 (100)

Fig. 1

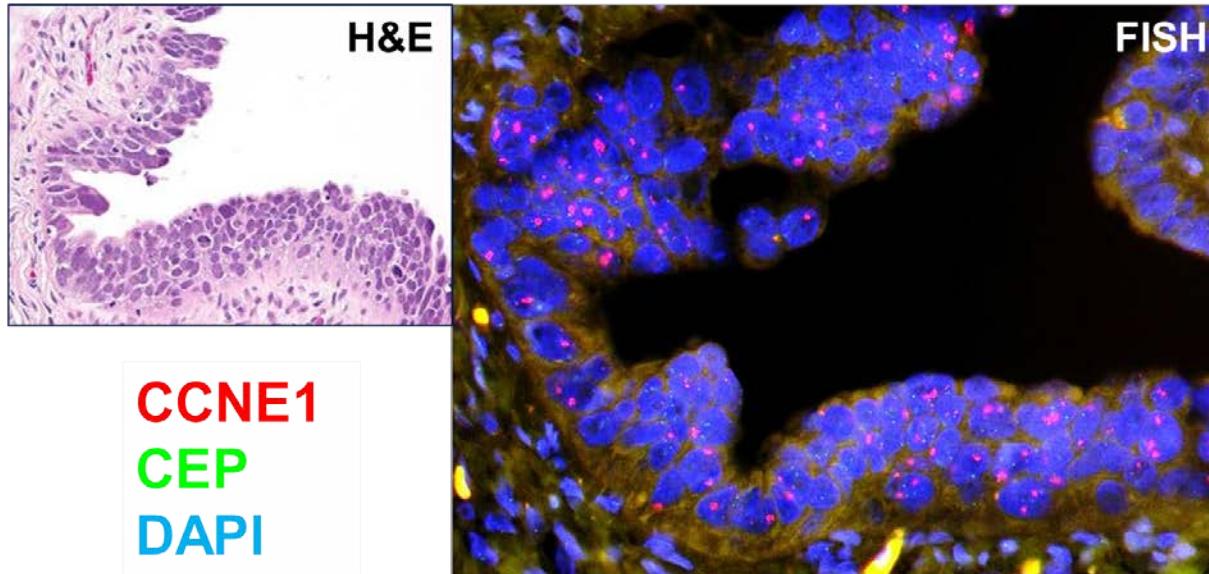


polysomy. For HGSCs, 12 (28%) of 43 HGSCs were *CCNE1* FISH positive, and they included 10 with amplification and 2 with high polysomy (Fig. 1 and Table 1). In this series, 30 STICs were associated with HGSCs, among which 11 were bifocal. We found a significant concordance in *CCNE1* copy number between STIC and HGSC from the same 19 patients ($p <0.001$). There was no significant difference in the percentage of *CCNE1* FISH positive cases between STICs and HGSCs ($p= 0.613$, Chi square) (Table 1). No evidence of *CCNE1* copy number changes was noted in normal-appearing fallopian tube epithelium adjacent or remote to STICs. A representative STIC showing *CCNE1* amplification is shown in Fig. 2. There were 20 HGSCs examined on whole sections and 23 HGSCs in tissue microarrays and there was a strong correlation in percentage of *CCNE1*

copy number changes between whole section and tissue microarrays ($r^2= 0.993$, $p < 0.05$), indicating that tissue microarray format was compatible with FISH analysis.

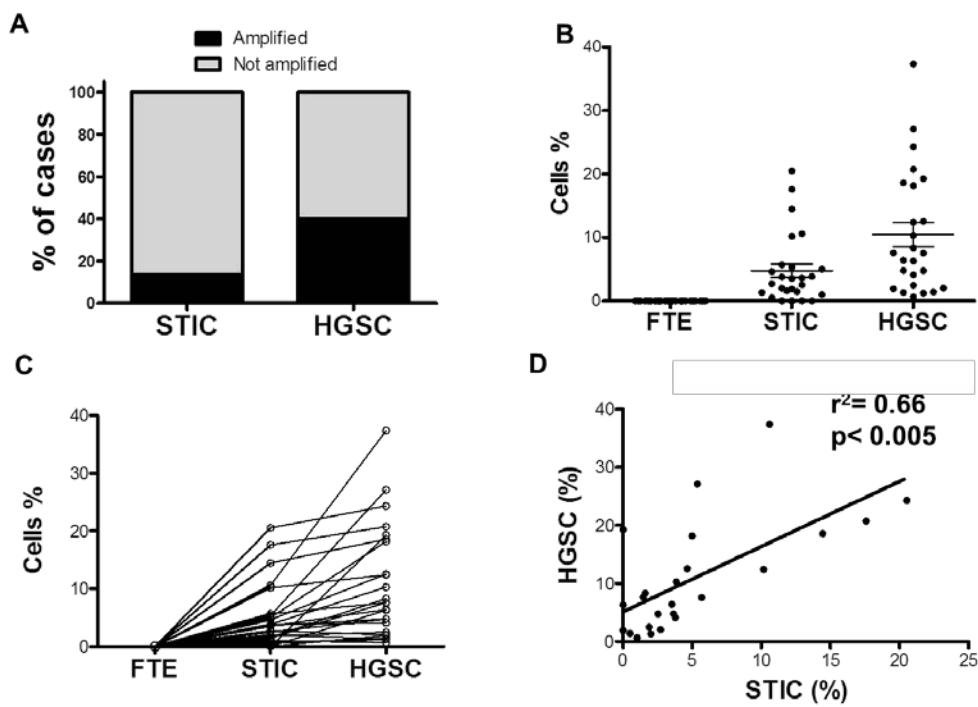
Next, we assessed centrosome amplification as defined by an increased number of centrosomes (> 2) in interphase tumor cells in both STICs and HGSCs. To measure the centrosome number, a double-color immunofluorescence for γ -tubulin and α -tubulin was performed to simultaneously

Fig. 2



visualize centrosomes and microtubules, respectively. Centrosome amplification was recorded in only 5 (14%) of 37 STICs, but in 10 (40%) of 25 HGSCs (Fig. 3A). Only whole sections were stained for centrosome

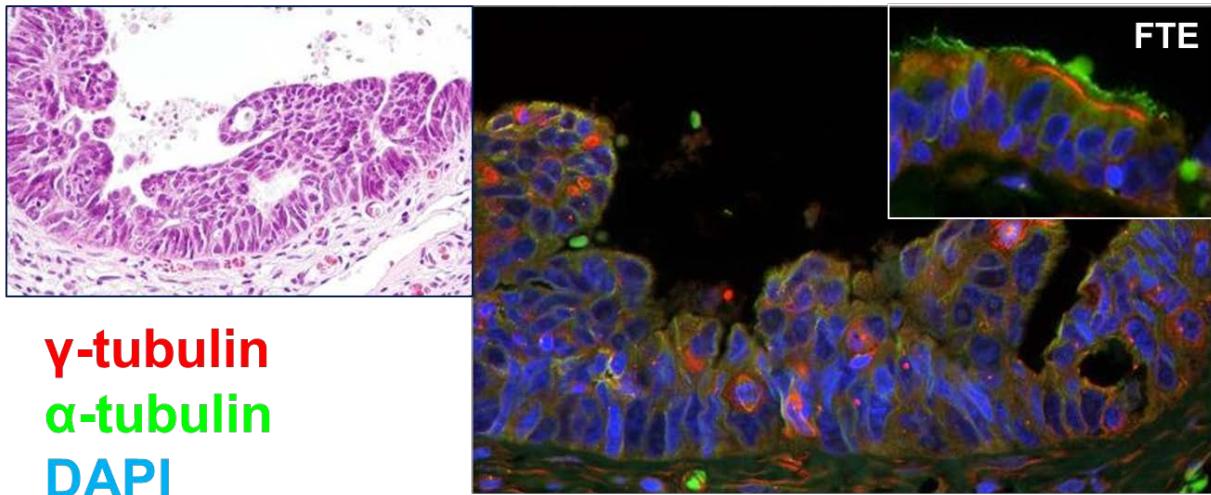
Fig. 3



immunofluorescence. There was a significant increase in the percentage of cells with centrosome amplification in HGSC as compared to STIC ($p = 0.0006$, Wilcoxon rank test) (Fig. 3B). A representative STIC showing centrosome amplification is illustrated in Fig. 4. A pair-wise comparison of normal fallopian tube epithelium, STIC and HGSC from the same cases demonstrated that 21 (88%) of 24 HGSCs had higher percentage of cells showing centrosome amplification than corresponding STICs

and three of the HGSCs demonstrated a mild decrease in percentage of cells with centrosome amplification as compared to the matched STICs (Fig. 3C). In general, there was a positive correlation in percentage of cells with centrosome amplification between HGSC and STIC from the same patients (Fig. 3D). We did not observe any abnormal centrosome number in normal-appearing fallopian tube epithelial or stromal cells.

Fig. 4



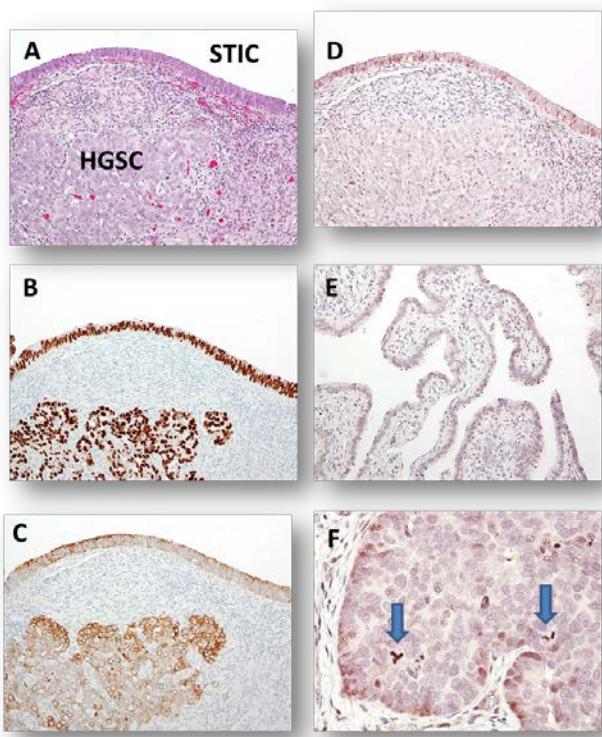
In summary, the findings from this study demonstrate that *CCNE1* copy number gain/amplification occurs in 22% of STICs that are associated with HGSCs, suggesting that amplification of *CCNE1* serves as one mechanism in developing some STICs. Moreover, centrosome amplification in tumor cells is more frequently detected in HGSCs than STICs, indicating a progressive acquisition of chromosomal instability during tumor progression and this finding may lend further support to the new paradigm that many of HGSCs may arise from STICs.

Task 2. Determine the early molecular changes associated with serous tubal intraepithelial lesions.

Task 2a. Immunohistochemistry study on ovarian cancer-associated markers on STICs and other putative precursor lesions (18-40 months)

Progress: This task has largely been completed. So far, we have identified at least 5 ovarian cancer-associated markers on STICs including LAMC1, CCNE1, topoisomerase II, RSF-1, and loss of

ALDH1A1. In this period of time, we have focused on validating another important cancer gene, called TET1 on STIC and HGSC. Our finding shows that TET1 is upregulated as early as at the stage of STIC in addition to HGSC (Fig. 5). We plan to publish this result together with other mechanistic findings in a separate manuscript for publication.



Task 2b. In situ hybridization and/or mRNA expression analysis on those markers that the antibodies are not available (30-40 months)

Progress: As discussed in the last report, we have been fortunate to identify good antibodies (laminin C1, ALDH1A1, p53, topoisomerase II, cyclin E1, etc.) for immunostaining purposes and there is no need for us to consider *in situ* hybridization at this moment.

Fig. 5. A: H&E B. p53 C. Laminin C1. D. TET1, E. TET1 on normal fallopian tube epithelium. F. TET1 immunoreactivity on HGSC higher magnification to show mitotic figures (arrows) are intensely positive.

Task 2c. Verification of new markers from Project 3 in precursor lesions (24-56 months)

Progress: As described previously in the Task 2a, we have verified several new markers in STICs including LAMC1, ALDHA1 and cyclin E1. The results have been reported in the past progress reports and will not be reiterated here. They have been or will be published in the following journals including J Pathol (PMID: 23378270), Am J Surg Pathol (PMID: 22892598) and Mod Pathology (PMID: 25216223).

Task 2d. Telomere FISH on STICs and other precursor lesions (36-48 months)

Progress: This part of study has been largely completed.

Task 2e. Data analysis and preparation for publications (40-60 months)

Progress: We are in the process of generating data although we continue publishing our results.

Section III. Problem Areas for Project 1

For task 1C, in our original proposal, we propose to use molecular genetic methods and markers such as allelic imbalance at specific subchromosomal loci and passenger mutations to show the difference of these features between STIC and HGSC. However, we have tried very hard to obtain sufficient genomic DNA isolated from the minute STIC but had no luck. Therefore, we seek for alternative approach. As a result, we have applied alternative method to accomplish the objective to show that STIC represents the precursor rather than metastasis of HGSC. We are able to demonstrate that STICs have shorter telomere length and lower degree of centrosome amplification than the adjacent high-grade serous carcinomas (HGSC). Therefore, our results support the tubal hypothesis of developing HGSC in many cases.

Section IV. Future Works in Project 1

Task 1b and 1c. TP53 mutational analysis of all the potential precursor lesions of HGSC and allelic imbalance assay by digital SNP analysis and data analysis.

In the next half an year, we will wrap up the CCNE1 and centrosome amplification paper and submit it for publication.

Task 2a. Immunohistochemistry study on ovarian cancer-associated markers on STICs and other putative precursor lesions.

In the next 6 months, we will focus on finishing the study related to the TET1 expression in a panel of HGSCs in tissue microarrays and a cohort of STICs. We will prepare this part of study for publication. *All tasks.* We will write a review paper that summarize our recent studies as supported by DoD and submit it to Am J Pathol for publication. The title and abstract of our new review paper will be as follows.

The Dualistic Model of Ovarian Carcinogenesis. Revisited, Revised and Expanded

Since our proposal of a dualistic model of epithelial ovarian carcinogenesis over a decade ago, a large number of molecular and histopathologic studies have been published that have provided important insights into the origin and molecular pathogenesis of this disease. This has required that the original model be revised and expanded to incorporate these findings. The new model divides type I tumors into three groups: 1) endometriosis-related tumors which include endometrioid, clear cell and seromucinous carcinomas, 2) low-grade serous carcinomas and 3) mucinous carcinomas and malignant Brenner tumors. As in the previous model, type II tumors are composed, for the most part, of high-grade serous carcinomas which can be further subdivided into morphologic and molecular subtypes. Type I tumors develop from benign extraovarian lesions that implant on the ovary and which can subsequently undergo malignant transformation while many type II carcinomas develop from intraepithelial carcinomas in the fallopian tube and, as a result, disseminate as carcinomas that involve the ovary as well as extraovarian sites, which probably accounts for their clinically aggressive behavior. The new molecular genetic data, especially those derived from next generation sequencing, further underscore the heterogeneity of ovarian cancer and identify actionable mutations. The dualistic model highlights these differences between type I and type II tumors which, it can be argued, describe entirely different groups of diseases.

Project 2: The relationship between serous tubal intraepithelial carcinoma and invasive pelvic serous carcinoma

1. INTRODUCTION:

Ovarian cancer has traditionally been thought to develop from the OSE or cortical inclusion cysts, but recent data suggest that a majority of advanced HGSC may originate from the fallopian tube epithelium. Although highly provocative, this hypothesis requires further validation and therefore we propose to analyze a large group of pelvic, which includes ovarian, tubal and primary peritoneal, HGSCs diagnosed using traditional criteria with and without STICs in women whose fallopian tubes have been processed using the SEE-FIM technique, currently the most comprehensive method of evaluating fallopian tube epithelium. Our primary objective is to determine whether there are subsets of HGSC, which have different molecular profiles and different clinical behavior based on their presumed site of origin or whether there are no differences and that they are essentially the same irrespective of their site of origin. We will also compare the molecular profiles of normal tissues to HGSCs as a whole and HGSCs with and without STICs placing specific emphasis on the ovarian surface and distal fallopian tube epithelium.

2. KEYWORDS: Serous tubal intra-epithelial carcinoma, ovarian cancer, ovarian carcinoma, high-grade serous carcinoma, serous, STIC, in-situ cancer, SEE-FIM, ovarian surface epithelium, fallopian tube, genomic profiling, molecular, genomics.

3. ACCOMPLISHMENTS:

MAJOR GOALS:

Task 1: Determine the frequency of STICs in patients with advanced pelvic HGSC.

Task 2: Evaluate whether the presence of a STIC is associated with different clinical manifestations and /or outcome compare to those patients in whom a STIC was not identified.

Task 3: Compare the molecular features of advanced pelvic HGSCs with and without associated STIC to various normal pelvic tissues.

ACCOMPLISHEMENTS UNDER THESE GOALS (FOR THIS REPORTING PERIOD):

Task 1: Complete – nothing to report for this period.

Task 2: Evaluate whether the presence of a STIC is associated with different clinical manifestations and /or outcome compare to those patients in whom a STIC was not identified.

Task 2a: Collect clinical data from specimens identified in Task 1a, above. (Completed previously)

Task 2b: Analyze clinical variables in the context of specimens with or without STIC

1. Major activity – analysis is complete
2. Specific objectives: To determine the frequency of the STICs in patients with advanced stage pelvic HGSC and evaluate whether the presence of a STIC lesion is associated with different clinical manifestations and / or outcome compared to those patients in whom a STIC was not identified.
3. Significant results: Three-hundred ninety-eight women with fallopian tube, ovarian, or primary peritoneal carcinoma were identified between 2009-2012. Two-hundred twenty-eight (57%) patients met all inclusion criteria. Median age of the population was 62.4 years (range, 32.2-86.9). Ninety-three (41%) cases had STIC lesions and 135 (59%) did not. Thirty-nine patients in this cohort had BRCA

germline mutations; 16 (32.7%) in patients with STIC lesions and 23 (31.1%) in patients without STIC. The majority of patients had stage IIIC disease (168, 73.7%) and received an optimal cytoreduction at time of their primary operation (207, 90.8%). Platinum-resistant disease was noted in 35 (17.1%) patients; 18 (21.4%) patients with STIC, and 17 (14.0%) in patients without STIC. Median follow-up among all patients in study was 26.9 months (range, 0.3-52.7). Of those with STIC lesions, 70 (75.3%) were reported in the original pathology report; 23 (24.7%) were identified upon study review. Of the patients without STIC, 25 (19%) had comprehensive tubal processing (SEE-FIM), 31 (23%) had representative tubal sections, 8 (6%) were tubal carcinomas without STIC lesions, and 79 (59%) are pending review. After pathologic re-review of cases that did not report or had not originally identified STIC lesions, an additional 23 (24.7%) cases with STIC lesions were identified. The number of primary fallopian tube carcinoma diagnoses doubled from 61 (26.8%) to 119 (52.4%) after pathology re-review due to the identification of these additional cases with STIC. Kaplan-Meier survival curves did not demonstrate a statistical difference in progression-free (PFS) and overall-survival (OS) when comparing cases with and without STIC lesions ($P=0.11$ and $P=0.37$, respectively). However, a trend of poorer PFS among patients with STIC lesions was observed.

Conclusions: STIC lesions are present in approximately 41% of cases with HGSC of pelvic origin. There are no differences in standard clinical parameters between STIC and non-STIC cases. There is a non-statistical increase in platinum-resistance and a corresponding decrement in progression-free survival for cases with STIC lesions. Further data is required to determine if this is a significant finding.

4. Other achievements: None to report

Task 3: Compare the molecular features of advanced pelvic HGSCs with and without associated STIC to various normal pelvic tissues.

Task 3a. Collect 100 HGSC specimens that had SEE-FIM processing from Consortium sites; 10-20 specimens per site with the balance contributed from MSKCC (Completed previously)

Task 3b. Collect 10 normal tissues from each of 5 anatomic sites; 50 total tissues. Tissues to be collected across Consortium sites, with each site contributing at least 2 of each normal tissue type with the balance contributed from MSKCC (Completed previously)

Task 3c. Process all specimens on various genomic platforms

All tumor specimens had been processed during previous reporting periods. During this past period, we have been able to finalize quality control (QC) on our normal tissues, which had been challenging due to small cellular quantities obtained from anatomical brushings. We were able to develop a pooling strategy such that at least 3 pools (of 3-5 patient specimens per pool) from each normal anatomic site generated sufficient material to perform RNA sequencing. The RNA sequencing is currently in progress.

Task 3d. Analyze data on each platform according to proposal.

1. Major activity – analysis of tumor samples is complete
2. Specific objectives: To better understand the molecular etiology of HGSCs, we report a multi-center integrated genomic analysis of advanced stage tumors with and without STIC lesions.
3. Significant results: (Results presented in prior reporting period) Unsupervised cluster analysis of miRNA data failed to identify a separation between cases with and without STIC lesions. The most significant focal DNA somatic copy number alterations were shared between cases with and without STIC lesions.

Class comparison of the RNA sequencing data using the 9,236 most variable genes identified 69 differentially expressed genes at $P < 0.01$, none of which passed multiple comparison correction.

Conclusions: The data suggest that the molecular features of HGSCs with and without associated STIC lesions are mostly shared, indicating a common biologic origin among all cases. We propose that HGSC originates in the distal fallopian tube and a STIC lesion is only identifiable in ~50% of cases prior to intraperitoneal shedding of malignant cells.

(New results for this reporting period) Since the last reporting period, we have been working on performing general validation analyses for the data and additional analyses on the overall dataset. We started by reproducing the established gene expression clusters for high-grade serous ovarian carcinoma as previously published and demonstrated in Figures 1 and 2 [PMID: 21720365, 18698038, 23257362]. Among these cases we did not see a difference in progression-free survival (Figure 3). We also identified multiple regions of focal amplification and deletion that appear similar between tumors with and without STIC lesions (Figures 4 and 5).

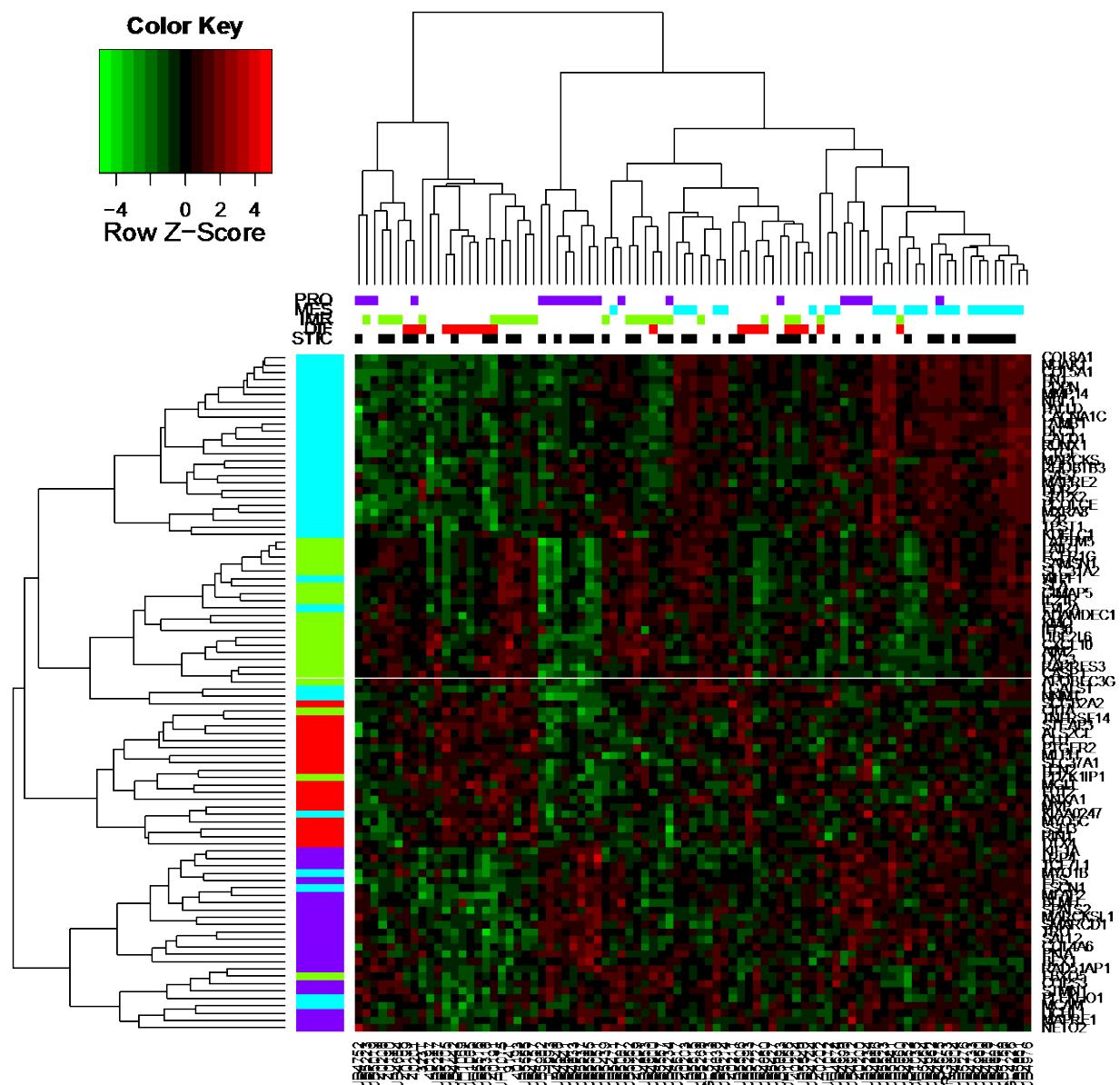


Figure 1. Expression Clustered using 100-gene subtype signature. Colors for subtypes in top bars were given to samples with greater than 0.2 correlation with each respective subtype. Row colors indicate assignment of genes to subtypes by Verhaak and Levine. Genes and samples clustered by correlation. Blocks of overexpression for the correlated samples and their respective subtype genes are visible in some cases. The gene subtypes clustered closer than the samples correlated with each respective subtype.

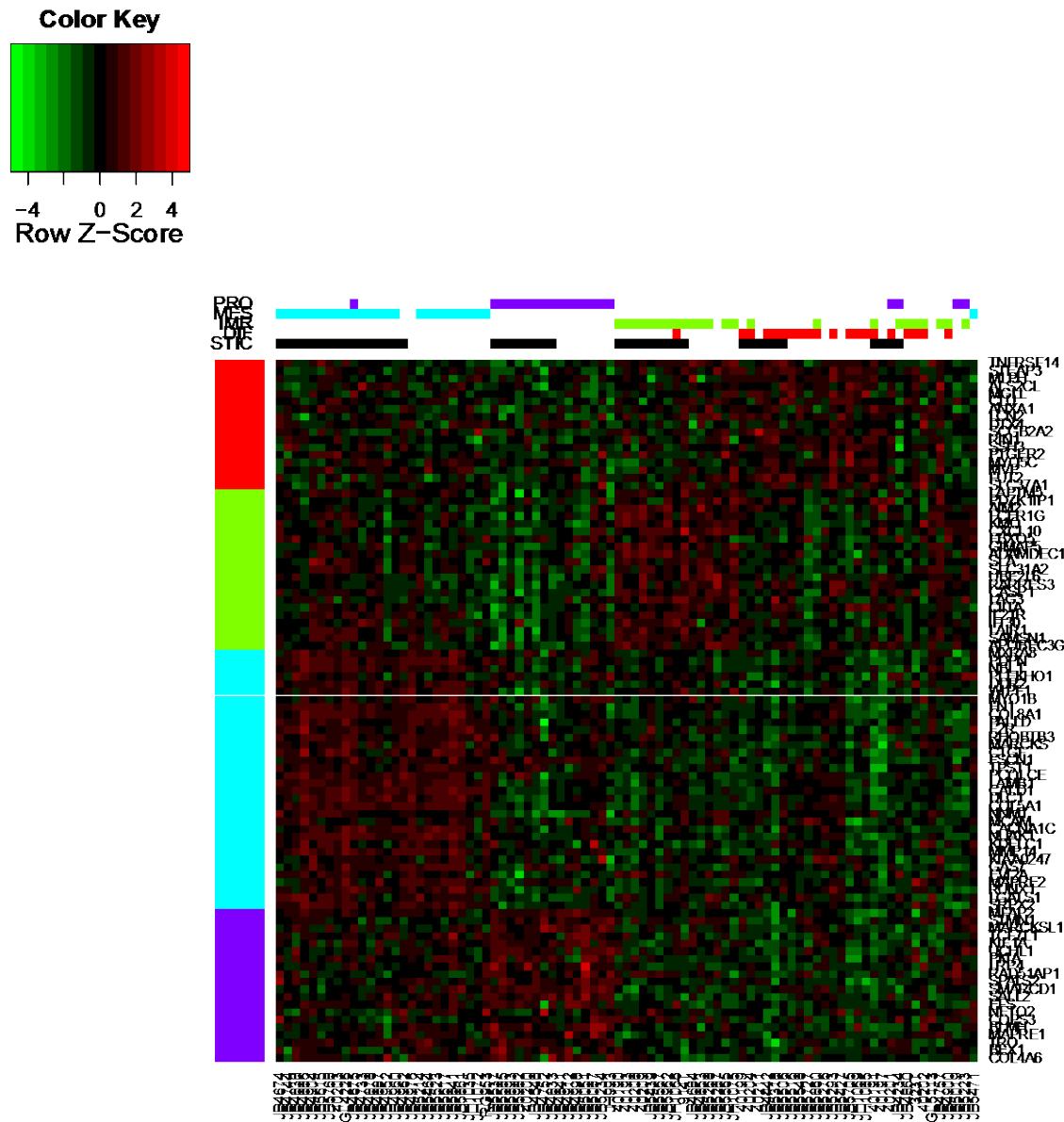


Figure 2. Expression Ordered by Assigned Subtype. Samples whose highest correlation was at least 1.5x higher than the next highest subtype correlation were assigned to that subtype. Colors for subtypes in top bars were given to samples with greater than 0.2 correlation with each respective subtype. Row colors indicate assignment of genes to subtypes by Verhaak and Levine. Genes and samples ordered by assigned subtype, the final group to the far right were not assigned to any particular subtype. The blocks of overexpression are clearly represented in the samples correlated to each respective subtype in the subtype genes. STIC status does not have a clearly defined effect.

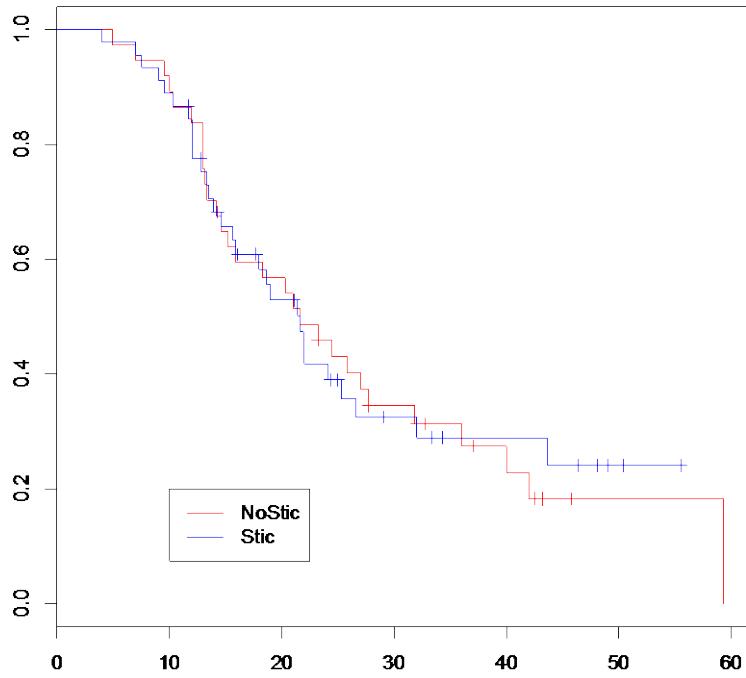


Figure 3. Progression Free Survival by STIC Status. The results are not statistically significant; $p=0.944$.

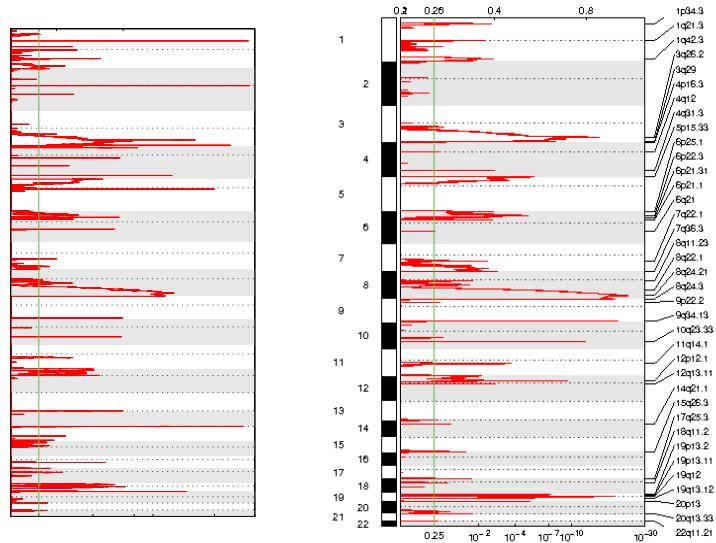


Figure 4. Significant Amplifications. Left panel: significantly amplified regions in tumors without identified STICS. Middle panel: significantly amplified regions in STIC associated tumors. Right panel: significantly amplified regions in all tumors. Results generating using Gistic2.0 after circular binary segmentation.



Figure 5. Significant Deletions. Left panel: significantly amplified regions in tumors without identified STICS. Middle panel: significantly amplified regions in STIC associated tumors. Right panel: significantly amplified regions in all tumors. Results generating using Gistic2.0 after circular binary segmentation.

Other achievements: None to report

TRAINING AND PROFESSIONAL DEVELOPMENT: Nothing to Report.

DISSEMINATION TO COMMUNITIES OF INTEREST: Results were presented at the 2015 Annual Meeting of the Society of Gynecologic Oncology.

PLANS DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH GOALS: We will complete any outstanding analyses on the tumors from Task 3. We will have submitted a manuscript by the next reporting period, as one is already in preparation. We will have completed the RNA sequencing of the normal samples by the next reporting period.

4. IMPACT:

IMPACT ON THE DEVELOPMENT OF THE PRINCIPAL DISCIPLINE: These results are expected to make an impact on the understanding of ovarian cancer tumorigenesis. The outstanding question in our principal discipline is whether or not all high-grade serous carcinomas develop from the distal fallopian tube through serous tubal intra-epithelial carcinomas. These data and results will support the notion that nearly all high-grade serous carcinomas do develop from the distal fallopian tube through serous tubal intra-epithelial carcinomas since little genomic variation has been found between tumors with and without serous tubal intra-epithelial carcinoma. These data will ultimately help to shape prevention and early detection approached for ovarian cancer.

IMPACT ON OTHER DISCIPLINES: Nothing to report.

IMPACT ON TECHNOLOGY TRANSFER: The data will affect the development of technology used for early detection of ovarian cancer considering that devices will need to be developed for interrogation of the distal fallopian tube.

IMPACT ON SOCIETY BEYOND SCIENCE AND TECHNOLOGY: The findings will help to educate the society about the origins of ovarian cancer and encourage women throughout the world to request bilateral salpingectomy (removal of both fallopian tubes) instead of bilateral tubal ligation as a

measure to prevent unwanted pregnancy. The data is also likely to result in a greater role for bilateral salpingectomy at the time of hysterectomy with ovarian preservation.

5. CHANGES/PROBLEMS:

CHANGES IN APPROACH: None noted.

ACTUAL OR ANTICIPATED PROBLEMS OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM: We had some delays in collecting and processing the normal tissue samples due to the limited cellular contents obtained from fresh intra-operative brushings. We were able to overcome this problem through a pooling approach that is statistically sound and eliminates batch effects among the normal samples.

CHANGES THAT HAD A SIGNIFICANT IMPACT ON EXPENDITURES: There was an accounting error at MSKCC that resulted in approximately \$130,000 being charged to this account during the wrong budgeting period. This was a result from a failure to properly encumber anticipated and approved charges. During this reporting period we have had to review all expenses from prior years and cost transfer money from other funds to cover this shortfall.

OTHER CHANGES: There were no other changes to any human subjects, vertebrate animals or biohazard concerns.

6. PRODUCTS:

PUBLICATIONS, CONFERENCE PAPERS, AND PRESENTATIONS: Abstract presentation at the 2015 Annual Meeting of the Society of Gynecologic Oncology.

OTHER PRODUCTS: Genomic data will be publicly deposited during the next reporting period.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS :

INDIVIDUALS WHO HAVE WORKED ON THE PROJECT: Douglas A. Levine, Maria Bisogna, Narciso Olvera, Fanny Dao – No change.

CHANGE IN THE ACTIVE OTHER SUPPORT OF THE PD/PI(S) OR SENIOR/KEY PERSONNEL: Douglas A. Levine received a leadership award from the DOD to serve as Assistant Dean of the Ovarian Cancer Academy.

W81XWH-15-1-0428 (OC140582) (PI: Levine)

Congressionally Directed Medical Research Programs 9/30/2015 - 9/29/2020

The Ovarian Cancer Academy: A Team-based Science Approach

This project will help to plan the scientific and leadership aspects of the Ovarian Cancer Academy. It will also assist all Academy members with project design, grant review and interpretation of results.

OTHER ORGANIZATIONS INVOLVED AS PARTNERS: Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS: Nothing to report.

9. APPENDICES: None.

PROJECT 3. Identification of molecular changes preceding STICs in FTE from high-risk women using in vitro and in vivo models

Investigators: PI - Shaw, Shih

Research site: University of Toronto

INTRODUCTION:

In this project we will determine if the expression profiles of anatomically high risk FTE (fimbrial) from women at high genetic risk (BRCA1 mutation carriers) differs from the FTE profiles from women at low risk of HGSC, and we propose that these changes may play key roles in the earliest events of serous carcinogenesis. To this end, we will use a molecularly defined system to sequentially express ovarian cancer-associated genes including those identified in this project into ovarian surface epithelium (OSE) as well as fimbrial FTE to determine a) if FTE is more prone to neoplastic transformation and b) if the FTE-derived tumors more closely simulate HGSC than OSE-derived tumors.

Recently described precursors of high-grade serous carcinoma, the p53 signature, a latent precursor, and serous tubal intraepithelial carcinoma (STIC), a pre-malignant precursor, occur most frequently at the distal and fimbriated end of the fallopian tube (FTE). We recently demonstrated that the FTE of BRCA1 mutation carriers, at genetic risk of HGSC, have altered signaling pathways compared to controls. A key question is whether the gene expression differences identified at the ampulla between BRCA1 and non- mutation carriers are similar to differences at the fimbria. This study determines the transcriptome profiles of normal fimbrial FTE and normal ampulla FTE which may lead to insight of why the distal end of the fallopian tube is preferentially predisposed to malignant transformation

Specific Aim 1. Detect and select genes differentially expressed in morphologically normal fimbrial FTE from women at high genetic risk of HGSC.

Specific Aim 2. Model alterations associated with normal FTE from high-risk women and STIC in vitro and in vivo.

BODY:

Task 1. Establish expression profiles of fallopian tube epithelium from BRCA1 mutation carriers and controls, and of serous cancers in mutation carriers.

Progress: To date, we have collected and processed over 200 formalin-fixed and/or cryopreserved cases of fallopian tube and fimbriae specimens. These cases include samples from BRCA1 and BRCA2 mutation carriers undergoing prophylactic surgery, patients undergoing debulking surgery for High Grade Serous Carcinoma, and patients undergoing salpingo-oophorectomy for non-malignant reasons. An integral aspect of Project 3 *Specific Aim 1* is to determine the relationship between hormonal response and BRCA mutation status in the normal fallopian tube epithelium. As a result, a significant effort has been placed on determining the menstrual status of samples collected – this included reviewing the endometrium of corresponding samples when available. We have completed histological reviews of fallopian tubes from BRCA1 and BRCA2 mutation carriers, along with matching controls and cancers and identified 84 cases that can be used for gene expression profiling. One of the key questions within this aim is whether the gene expression differences identified at the ampulla between BRCA1 and non-BRCA1 mutation carriers is similar to differences at the distal end of the fallopian tube – the fimbria. We have determined therefore, to answer the ampulla versus fimbria conundrum, it would be technically robust to use cryopreserved ampulla and fimbria from non-BRCA mutation carriers with known ovulation cycle status.

A). Micro-dissection of selected cryopreserved tissue samples

Background: Recently described precursors of high-grade serous carcinoma (HGSC), the p53 signature, a latent precursor, and Serous Tubal Intraepithelial Carcinoma (STIC), a pre-malignant precursor, occur most frequently at the distal and fimbriated end of the fallopian tube (FTE). In 3 previous reports, we have demonstrated that the FTE of BRCA1 mutation carriers, at genetic risk of HGSC, have altered signaling pathways compared to controls. Ovarian production of ROS is released after the LH surge to induce ovulation. Reactive oxygen species (ROS) have been implicated in serous carcinogenesis. The objective of this study is to compare the transcriptome profiles of normal fimbria (high-risk epithelia prone to transformation) FTE and normal ampulla (low-risk epithelia) FTE which may lead to further elucidating why the distal end of the fallopian tube is the preferential anatomic location of the fallopian tube for malignant transformation.

Methods: Snap-frozen matched fimbria and ampulla tissues were controlled for age and ovarian cycle status. Cases included 12 luteal phase and 12 follicular phase samples from women at no known risk for ovarian cancer. Laser capture microscopy was used to micro-dissect FTE cells, using 7-10 sections per case. Total RNA was isolated, RNA extracted and cDNA amplified. The expression profiles were generated using Affymetrix Human Genome HTA-2.0 Array. 5 μ m sections of the FFPE specimen of the profiled cases were stained for Ki67, p53, CK7 and GSTA2

Results: Using gene level differential expression analysis with the Affymetrix Expression Console software, we performed unsupervised hierarchical clustering analysis with all 24 samples. We used a fold change of < -2 or > 2 and ANOVA adjusted p-value < 0.05 as a cut-off criteria for selecting genes. The cases clustered predominantly by ovarian cycle status rather than by their differences in anatomical origin or their matched pair. There were 427 genes differentially expressed amongst the 4 groups – Fim-Luteal, Fim-Follicular, Amp-Luteal and Amp-Follicular. Independent of ovarian cycle status, very few differences (65 genes – SALL1, ENOX1, ANXA13, PDK4, ME1, GSTA1, GSTA2 – genes involved in metabolic pathways) were observed between the ampulla and fimbria FTE.

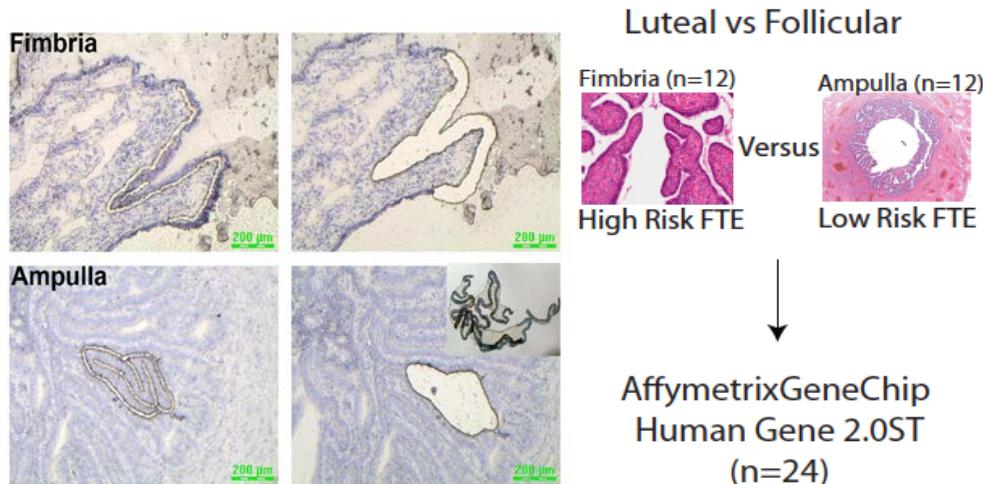


Figure 1: Cryopreserved fimbria and ampulla from fallopian tubes were sectioned and underwent laser-capture micro dissection. Epithelial cells were captured and RNA was isolated. Cases were matched by ovarian cycle status and were all obtained from pre-menopausal women.

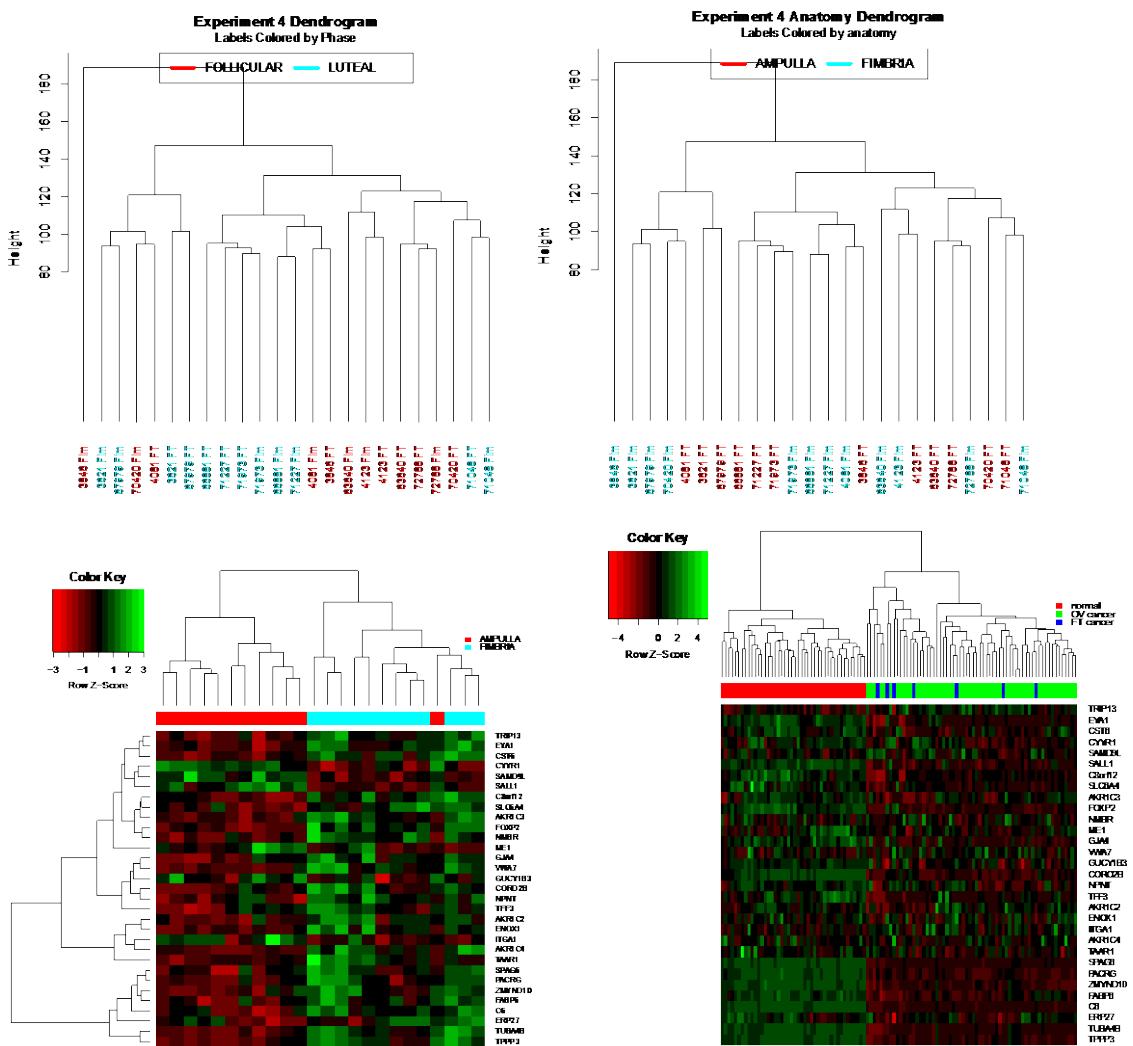
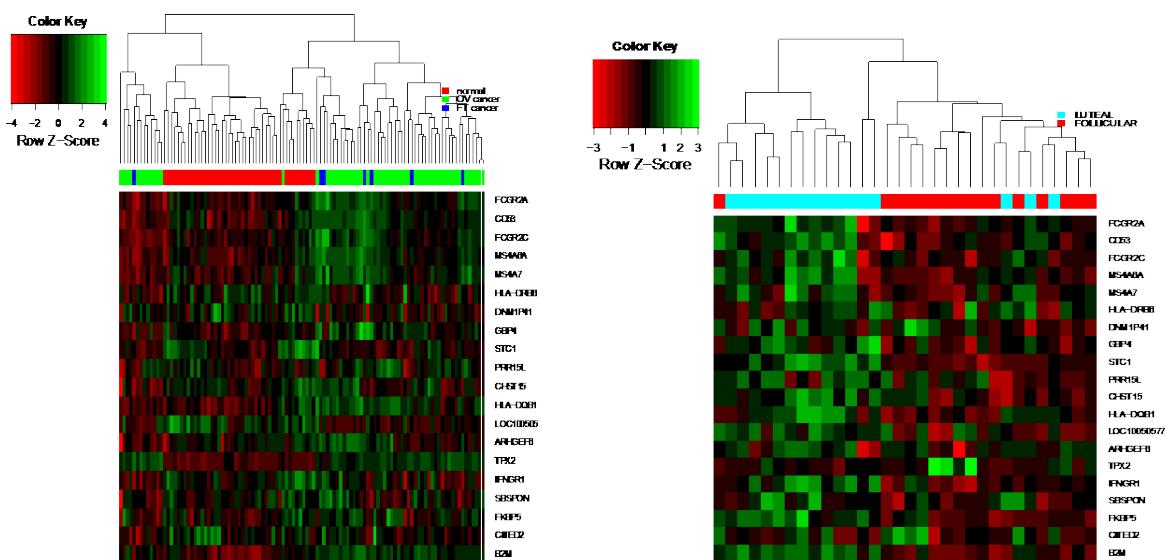


Figure 2: RNA samples were cDNA converted and run on the AffymetrixGene Chip Human 2.0ST array. **A-B** Dendograms of unsupervised hierarchical clustering data, with the labels colored by phase and anatomy. **A.** Heat map of supervised hierarchical clustering revealed significant differences in gene expression between fimbria and ampulla. **B.** 33 top ranked differentially expressed genes were used to determine cluster patterns in previously analyzed normal FTE and HGSC – ovary and fallopian tube. The cases (normal FTE, n=45, HGSC, n=56) separated into 2 major nodes claves – normal cells and carcinoma.

Figure 3: Heat map of supervised hierarchical clustering revealed significant differences in gene expression between follicular and luteal phases. The top differentially expressed genes (A) were used to interrogate normal FTE and HGSC of the ovary and fallopian tubes. As seen previously, some HGSC cases segregate by phase away from the normal FTE expression pattern.



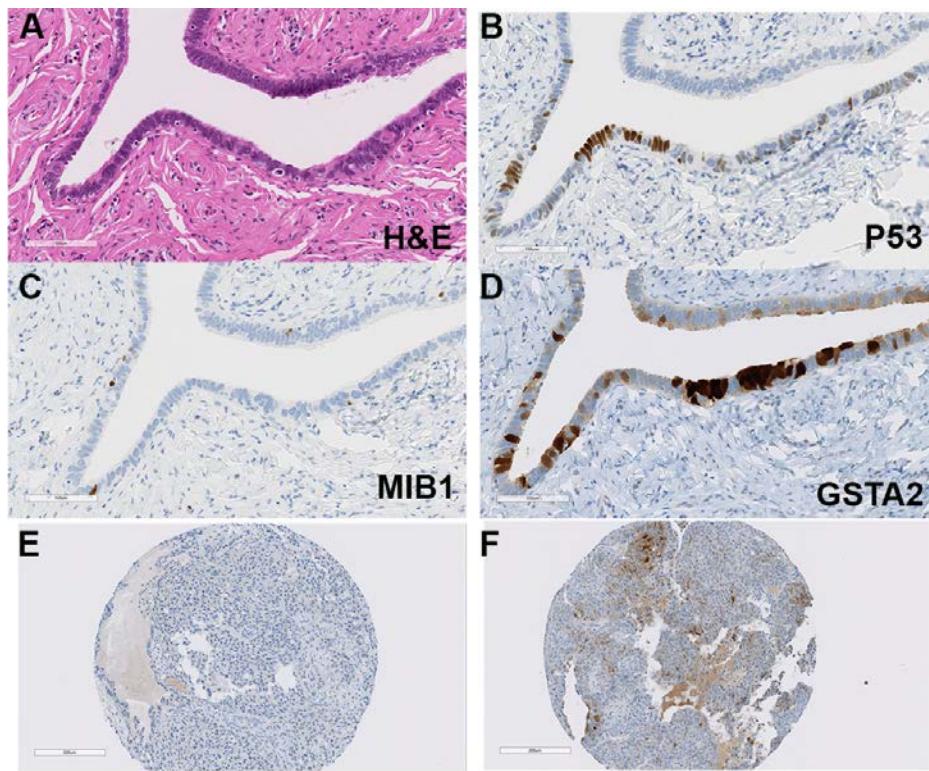


Figure 4: A-D. Decrease in expression of GSTA2 in a fallopian tube epithelial cells which over-express p53 (identified in a non-BRCA follicular phase sample) Expression of GSTA2 in the fimbria of human FTE. There are more GSTA2 positive cells in the fimbria compared to the ampulla. **E-F.** Expression of GSTA2 in high-grade serous cases is markedly reduced.

	Phase	N	Mean % +ve cells	Std. Deviation	Sig. (2-tailed)
					GSTA2
	Luteal	36	46.2	13.95868	0.203
	Follicular	32	41.2	18.12157	
	Anatomy	N	Mean % +ve cells	Std. Deviation	Sig. (2-tailed)
					GSTA2
	Fimbria	24	52.4	10.16764	0.001
	Ampulla	35	41.0	13.50871	

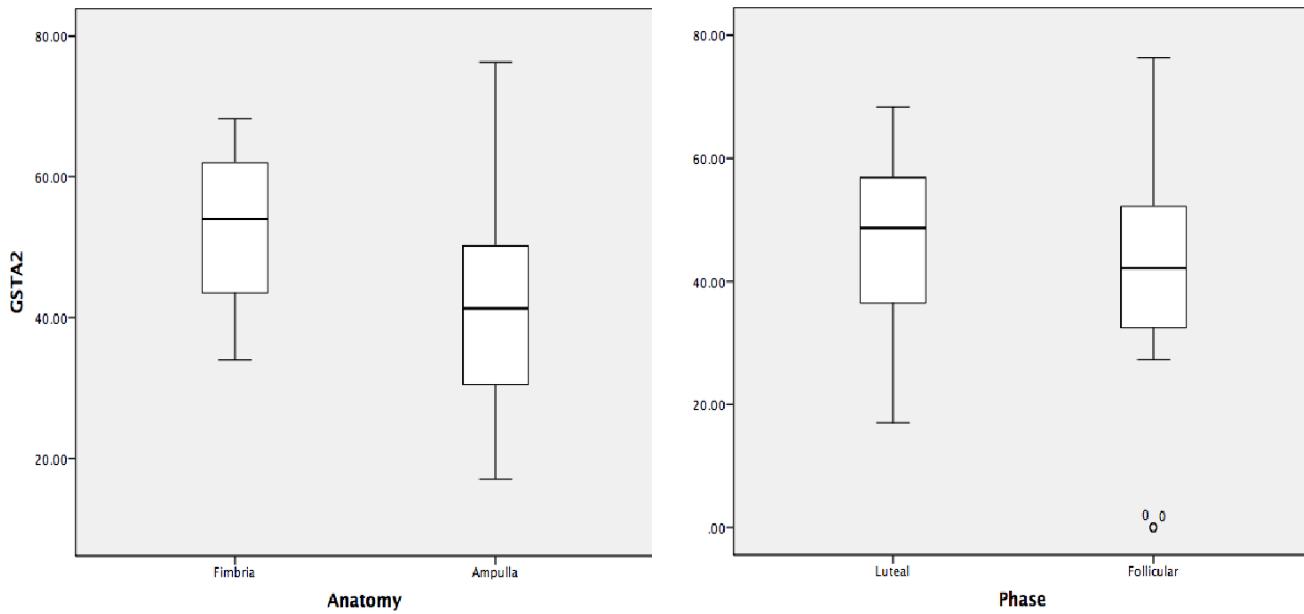


Figure 5: A. 3 independent observers scored the number of positive GSTA2 positive cells in the fimbriae and ampullae of 13 cases, multiple sections per cases, and 3 different regions of each anatomical area were scored (no. of +ve cells/100). The fimbria had significantly more GSTA2 expressing cells than the ampulla (T-test, $p=0.001$).

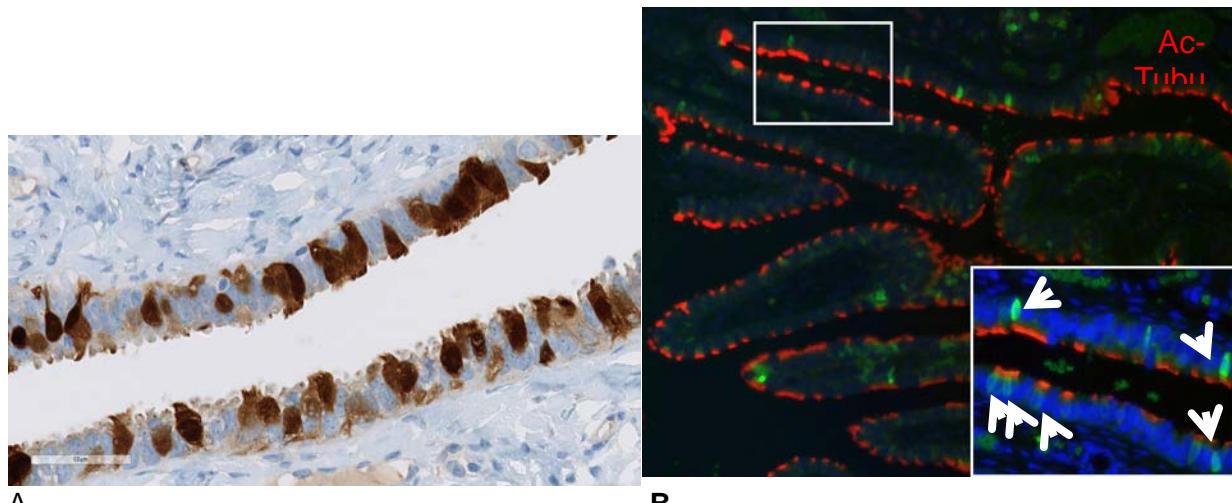


Figure 6: A. Expression of glutathione S-transferase A2 (GSTA2) seemed to be exclusively expressed in ciliated cells. Immunofluorescence of the fimbria. **B.** Acetylated-tubulin marks ciliated cells (red), while GSTA2+ cells (green) are expressed exclusively in ciliated cells (white arrow).

Discussion: The epithelia of the anatomically high-risk fallopian tube – the fimbria, show few differences in gene expression profiles compared to the lower risk portion – the ampulla. Expression differences predominantly are in response to the hormonal milieu, i.e. the follicular and luteal phases of the ovarian cycle. Distinct differences in differential expression of genes between the luteal and follicular phase are in part dependent on anatomic site. The changes are hormonally driven however, the response to the cycle changes are different in the epithelia located in the fimbria versus the ampulla. The increased anatomic risk of the fimbria is likely due to effects of the microenvironment, such as repeated exposure to follicular fluid at ovulation, rather than intrinsic differences of the FTE in the two sites. The ciliated cells uniquely express anti-oxidant enzymes (GSTA2) which is more abundant in the fimbria than the ampulla. Probed further, this insight will help understand the pre-neoplastic responses to ovulatory stresses in the fallopian tube epithelial cells.

B). Microdissection of selected paraffin tissue samples

We have completed laser capture micro-dissection (LCM) on fimbriae from FFPE (SEE-FIM) out of the proposed 71 cases; we have performed LCM on 71 cases which include 9 HGSC cases. These samples are ready for submission for the Illumina DASL arrays. We project the submission of all samples to the microarray facility within the next 2 months. Below is a summary of FFPE cases that have had LCM performed. Refer to Appendix 1 for a complete list of cases and RNA concentrations.

	# of cases	# cases with RNA extracted	# cases with RNA analyzed
BRCA Luteal	13	12	3
BRCA Follicular	23	22	5
Normal Luteal	11	8	1
Normal Follicular	13	12	1
HGSC BRCA1 Cancer	9	9	9
Post-Menopausal	2	2	0
Total	71	65	19

BRCA mutation	Ovulatory Phase
BRCA1	Follicular (n=7)
BRCA1	Luteal (n=3)
BRCA1 - 5382insC	Luteal (n=2)
BRCA1 - 5382insC	Follicular
BRCA1 1806C-T	Follicular
BRCA1 185delAG	Luteal (n=8)
BRCA1 185delAG	Follicular (n=3)
BRCA1 3533 INSG mutation	Follicular
BRCA1 4239 delAG	Follicular
BRCA1 917delTT	Follicular
BRCA1 g. 56490A>G	Follicular
BRCA1 IVS13- 3A-G	Follicular
BRCA1 IVS4-1 G-T	Luteal
BRCA1 NA	Follicular
BRCA1-1294del40 2434T-C variant	Follicular

Table 4: Distribution of BRCA mutation type across FFPE LCM Fimbria cases.

Task 2. Validate mRNA and protein expression of selected classifier genes.

Progress: In preparation for targets from the genomic profiling of STICs and fimbria gene expression arrays, we are in the process of creating one additional cancer TMA containing a large set of 300 HGSC samples with known family history of breast/ovarian cancer and patient clinical history including debulking status, treatment, recurrence and overall survival. This TMA will be useful in assessing the alteration of chosen targets within a larger set of cancers with different family history of cancer, as well as assessing the impact of such targets on clinical outcome. As the microarray analysis on the FFPE samples is in progress, the validations of targets in

both normal FTE and STIC are delayed. We anticipate completion of the gene expression experiments within 2 months.

Task 3. Model alterations associated with high-risk tubal epithelium and tubal intraepithelial carcinomas, in vitro.

Immortalized and transformed cell lines have been generated from both FTE and OSE, cells derived from BRCA mutation carriers and non-carriers. In particular, 16 OSE cell lines have been created – 3 from BRCA carriers – iOSE390F, iOSE267F, iOSE592F (F=familial ovarian cancer) and non-carriers – iOSE120 and iOSE523. Cell lines have been transfected with hTERT (ht) and SV40, and either vectors over-expressing cMYC, hRASV12 or PIK3CA-H1047R were generated.

In vitro assays were performed to compare proliferation, anchorage independent growth and invasion between 2 BRCA1 (iOSE 267F and iOSE 592F) and 2 non-BRCA cell line over expressing hTERT and SV40 and either cMYC or hRASV12. All 12 lines showed proliferation and anchorage independent growth.

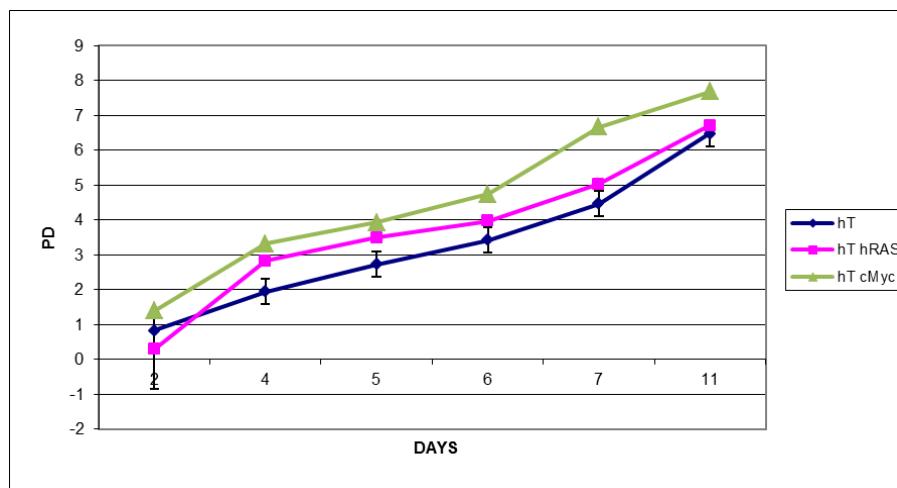


Figure 7: iOSE 120 SV40 htert cell line growth curve performed in-vitro. Experiment conducted over 11 days.

Eight of these lines with c-MYC or hRASV12 were then injected in NSG mice (NOD.Cg-Prkdcscid). All injections were performed in the mammary fat pad of 6 week old female mice. We are currently in the process of monitoring the mice and performing regular measurements of tumors that arise. Similarly, 16 FTE lines were generated from BRCA2 carriers - FTE3313 and FTE3798 and non-BRCA carriers – FTE3437 and FTE3619 over expressing hTERT and SV40 and either c-MYC or hRASV12. As with iOSE lines, 8 of the FTE lines have been injected in the mammary fat pad of NSG mice and we are currently monitoring the mice for tumor development.

Table 5: Summary of cell lines used to perform mouse injections to date.

Cell Line	Date of Injection
iOSE 120 SV40 hTERT hRAS v12	10-Jul-15
iOSE 120 SV40 hTERT cMYC	10-Jul-15
iOSE 267F SV40 hTERT hRAS v12	10-Jul-15
iOSE 267F SV40 hTERT cMYC	10-Jul-15

iOSE 523 sv40ht cMYC	24-Jul-15
iOSE 592f sv40ht cMYC	24-Jul-15
iOSE 523 sv40ht hRAS V12	6-Aug-15
iOSE 592f sv40ht hRAS V12	6-Aug-15
FTE 3437 SV40ht hRAS V12	4-Sep-15
FTE 3437 sv40 ht cMYC	4-Sep-15
FTE 3313 sv40ht cMYC	4-Sep-15
FTE 3798 SV40 hTERT hRAS V12	18-Sep-15
FTE 3798 SV40 hTERT + cMYC	18-Sep-15
FTE 3313 SV40ht hRAS V12	18-Sep-15
FTE 3619 SV40ht Hras v12	19-Oct-15

Project 5: Epidemiologic and molecular characterization of putative precursor lesions in the ovary and fallopian tube of unaffected high-risk women.

Specific Aim 1: Using existing samples (collected both in a retrospective and prospective manner) we will; a) determine the prevalence, frequency and location of STICs, STILs, p53 signatures, CICs, morphological changes in the OSE and invasive HGSCs in the FTE and ovaries of BSO specimens removed prophylactically in women at high risk for ovarian cancer, overall and stratified by BRCA1/2 mutation status (N ~ 550), b) examine correlations between the prevalence of the different types of lesions; and c) determine the epidemiological profile including demographics, lifestyle factors and known risk factors for ovarian cancer associated with STICs, STILs, p53 signature, CICs and morphological changes in the OSE.

*The Toronto group was responsible for the cutting, staining and shipment of slides from **prophylactic salpingo-oophorectomy specimens and 25 high-grade serous ovarian cancer cases**. A significant amount of effort has been devoted over the last 6 months toward the completion of this task due to time spent labeling slides, barcode scanning slides, organization into boxes and inventory creation of slides within their respective boxes. We have completed this task of the project with 189 prophylactic salpingo-oophorectomy cases and 25 HGSC cases. We have processed 1 HGSC case to be used in Project 1 as requested.*

KEY RESEARCH ACCOMPLISHMENTS:

- The epithelia of the anatomically high-risk fallopian tube – the fimbria, show few differences in gene expression profiles compared to the lower risk portion – the ampulla. Expression differences predominantly are in response to the hormonal milieu, i.e. the secretory and proliferative phases of the ovarian cycle.
- While the transcriptome changes in tubal epithelium are predominantly related to ovarian cycle changes, the response of fimbrial and ampulla cells do differ, and it is likely that responses to ovulatory follicular fluid at the fimbriated end may be in part responsible.
- The anti-oxidant enzyme GSTA2 has higher expression in the fimbrial cells compared to the ampulla, and is detected only in ciliated cells. This insight will help elucidate the pre-neoplastic responses to ovulatory stresses.
- Laser capture microdissection of 63 FFPE samples has been completed, extracted RNA submitted and the transcriptome analysis of the fimbrial FTE from BRCA carriers and non-carriers is in progress.
- We have generated immortalized and transformed OSE and FTE cell lines from BRCA mutation carriers and non-carriers harboring SV40 and hTERT along with hRASV12, PIK3CA-H1047R or cMYC and are currently performing *in vitro* studies. Some of the transformed cell lines have to date resulted in tumour formation when injected into the mammary fat pads of NSG mice.
- Submission of slides from 189 prophylactic salpingo-oophorectomy cases have been submitted. (Project 5)

REPORTABLE OUTCOMES:

Publications

1. Sophia HL George, Anca Milea, Ramlogan Sowamber, Alicia Tone, Rania Chehade and Patricia Shaw. Loss of LKB1 Protein Expression is Frequent in Serous Carcinoma. *Oncogene*, (23 March 2015) | doi:10.1038/onc.2015.62
2. Venkatesh Krishnan, Robert Clark, Marina Chekmareva, Amy Johnson, Sophia George, Patricia Shaw, Victoria Seewaldt, Carrie Rinker-Schaeffer. In vivo and ex vivo approaches to study ovarian cancer metastatic colonization of milky spot structures in peritoneal adipose. *Jove* 2015 10/14/2015, Issue 104; doi: 10.3791/52721
3. Lheureux S, Shaw PA, Karakasis K, Oza AM. Cancer precursor lesions in the BRCA population at the time of prophylactic salpingo-oophorectomy: Accuracy of assessment and potential surrogate marker for prevention. *Gynecol Oncol* 2015 Aug;138(2):235-7. doi: 10.1016/j.ygyno.2015.06.014. Epub 2015 Jun 10

Book Chapter

1. Patricia Shaw, Blaise Clarke and Sophia HL George. Precancerous lesions of high-grade pelvic serous carcinoma. Precancerous lesions of the gynecologic tract: diagnostic and molecular pathology. *In press* Springer 2015

Abstracts

1. George, S., Milea, A., Salman, N., & Shaw, P. (2015, February). Fimbria and Ampulla Tubal Epithelium Have Similar Transcriptome Profiles. *Laboratory Investigation* vol. 95, pp. 286A-286A, 2015.
2. Kolin, D., George, S., Milea, A., Narod, S., Clarke, B., & Shaw, P. (2015, February). The Familial Ovarian Tumor Study: A Morphological and Immunohistochemical Review. *Laboratory Investigation* vol. 95, pp. 293A-293A, 2015.
3. Peerani, R., George, S., Sowamber, R., Siu, A., Shao, T., Milea, A., ... & Shaw, P. (2015, February). Tumor Infiltrating Lymphocytes (TILs) as a Function of Histological Subtype and Genetic Background of Ovarian Epithelial Carcinomas. *Laboratory Investigation* vol. 95, pp. 301A-301A, 2015.
4. George, S., Milea, A., Sowamber, R., Considine, M., Salman, N., Chavin, N., Maamir, Z., Cope, L., Shaw, P. Transcriptome analyses of human fimbria and ampulla highlight similarities and differences in the human fallopian tube. AACR: Advances in Ovarian Cancer Oct, 2015.

CONCLUSIONS:

Through the work over the last 6 months we have begun to understand the biological relevance of genes found to be differentially expressed between BRCA1 and controls in the fallopian tube epithelium. Although still preliminary, we identify C/EBP-delta as a possible regulator of mesenchymal to epithelial transition in the fallopian tube epithelium. As a result of the work presented in the last report and abstract attached we are in the process of writing a manuscript for peer-review publication. Additionally, the work on estrogen and progesterone receptor signaling in the FTE, STIC and HGSC is being prepared for peer-review publication. Our team continues to work on the modeling of alterations in BRCA mutation carriers in *in vitro* and *in vivo* models, and have successfully demonstrated tumor growth in mice from transformed human cell lines. We will present data of this work, including preliminary analysis of tumours formed, in the next report.

We have also demonstrated that the fimbria and ampulla of the tube are very similar at the transcriptional level. We therefore stipulate that the higher-risk area of the tube - the fimbria, is more exposed to DNA damage and effects from the ovulation due its proximity to the ovary. Additionally, we are awaiting transcriptome analysis of the microdissected fimbrial FTE from mutation carriers. Preliminary analysis will be presented in the next report. We continue to study at the immunohistochemical level, the differences between the histological normal FTE and that in BRCA mutation carriers, using targets obtained from the previous microarray studies.

APPENDIX:

1. Sophia HL George, Anca Milea, Ramlogan Sowamber, Alicia Tone, Rania Chehade and Patricia Shaw. Loss of LKB1 Protein Expression is Frequent in Serous Carcinoma. *Oncogene*, (23 March 2015) | doi:10.1038/onc.2015.62.
2. Lheureux [S](#), [Shaw PA](#), [Karakasis K](#), [Oza AM](#). Cancer precursor lesions in the BRCA population at the time of prophylactic salpingo-oophorectomy: Accuracy of assessment and potential surrogate marker for prevention. *Gynecol Oncol* / 2015 Aug;138(2):235-7. doi: 10.1016/j.ygyno.2015.06.014. Epub 2015 Jun 10
3. George, S., Milea, A., Salman, N., & Shaw, P. (2015, February). Fimbria and Ampulla Tubal Epithelium Have Similar Transcriptome Profiles. *Laboratory Investigation* vol. 95, pp. 286A-286A, 2015.

4. Kolin, D., George, S., Milea, A., Narod, S., Clarke, B., & Shaw, P. (2015, February). The Familial Ovarian Tumor Study: A Morphological and Immunohistochemical Review. *Laboratory Investigation* vol. 95, pp. 293A-293A, 2015.
5. Peerani, R., George, S., Sowamber, R., Siu, A., Shao, T., Milea, A., ... & Shaw, P. (2015, February). Tumor Infiltrating Lymphocytes (TILs) as a Function of Histological Subtype and Genetic Background of Ovarian Epithelial Carcinomas. *Laboratory Investigation* vol. 95, pp. 301A-301A, 2015.
6. George, S., Milea, A., Sowamber, R., Considine, M., Salman, N., Chavin, N., Maamir, Z., Cope, L., Shaw, P. Transcriptome analyses of human fimbria and ampulla highlight similarities and differences in the human fallopian tube. AACR: Advances in Ovarian Cancer Oct, 2015.

Project 4. Locate and characterize precursor lesions of “ovarian” cancer in a mouse model and explore the role of ovulation and changes in the microenvironment of the ovary and tube in “ovarian” carcinogenesis using human tubal xenografts in nude mice.

Research site: Johns Hopkins University

Project Leader: Tian-Li Wang

Co-investigators: Ie-Ming Shih (JHU)

Section II. Progress to Date

Task 1. Locate the anatomic site and characterize the precursor lesions of “ovarian” cancer in the TP53^{-/-}/Rb^{-/-} mouse model.

Task 1a. Mouse breeding and genotyping (1-18 months)

Progress: This task has been finished. Please see the following tasks for details.

Task 1b. TP53/Rb KO mice study to locate the anatomic site of precursor lesions (1-15 months).

Progress: As discussed in the previous progress reports, we have finished the study related to TP53/Rb KO mice but failed to observe tumor phenotypes in at least 20 female mice for a period of time up to 16 months. Therefore, we have sought to use another mouse model. To this end, we have focused on Ovgp-1 Tag mouse model in which the expression of T antigen oncogene is driven the promoter of oviduct glycoprotein 1 that is specifically expressed by müllerian ductal tissues. Since large T antigen simultaneously inactivates both Rb and p53 pathways, we consider it as a good model to study ovarian serous carcinomas in which mutations and deletions are frequently observed. In fact, we have recently published the characterization of this model and demonstrate that this model is very useful for Project 4 (please see below). The Ovgp-1 mouse ovarian cancer model has been published (J Pathol. 2014 Jul;233(3):228-37).

Task 1c. Characterize the mouse precursor lesions using a variety of proposed methods (10-33 months).

Progress: This task has been completed and the data has been published and presented in the last progress report and will not be re-iterated in the current report. *J Pathol*, 2014. PMID:24652535

Task 2. Assess the biological effects of ovarian follicular fluid on human fallopian tube epithelium (FTE) and ovarian surface epithelium in a xenograft model.

Task 2a. Establish the human fallopian tube xenograft model (18-30 months).

Progress: We have finished exploring this model for the purpose of ovarian cancer research in mice and the results were shown in the previous progress report (with a figure illustration). So, the results will not be reiterated herein.

Task 2b. Collect human follicular fluid and primary characterization of the fluid (15-25 months).

Progress: This task is in progress. So far, we have obtained two batches of follicular fluids, FT406 and FT2826. Each batch came from 6 women whose follicular fluids were pooled. We have used these two batches of follicular fluids for initial characterization (see the following Task 2C).

Task 2c. Assess the biological effects of ovarian follicular fluids on FTE and OSE in a xenograft model (30-48 months).

Progress: As described in the previous reports, we have collected human follicular fluids and human tubal fluids and tested their DNA damaging effects on FT cultures. As described in the last progress report, we have performed the comet assay and gH2A staining and Western blot analyses to quantitate the damages in double strand DNA. Application of follicular fluid to the mouse xenograft model (Task a) appears challenging as the fluid may not penetrate into the grafted fallopian tube epithelium which is embedded by fibrotic tissues. Alternatively, we are considering an alternative study to expose the fresh

human fallopian tube to the fluid and analyze DNA damage levels after exposure at different time points.

Task 3. Determine whether oral contraceptives (OCPs) and NSAIDs reduce the morphologic and molecular changes that are associated with early “ovarian” carcinogenesis.

Task 3a. To assess whether OCPs decrease the frequency of precursor lesions and/or delay tumor development (24-48 months)

Progress: We have finished this task and found that there was no difference in the experimental and control groups after exposure of OCPs.

Task 3b. To determine the effects of aspirin on reducing oxidative stress-induced molecular changes on human fallopian tube and/or on OSE (24-60 months).

Progress: As discussed in the last progress report, we have changed our focus on studying two prescribed drugs including statin and bisphosphonate and determine if they have chemoprevention effects on the development of STIC and HGSC using the OVGp1 mouse model. In the previous 6 months, we finished the study on statin and have very recently published the paper in Clin Cancer Res 2015 Oct 15;21(20):4652-62. We demonstrate that lovastatin significantly reduced the development of STICs in mogp-TAG mice and inhibited ovarian tumor growth in the mouse xenograft model.

Knockdown of prenylation enzymes in the mevalonate pathway recapitulated the lovastatin-induced antiproliferative phenotype. Transcriptome analysis indicated that lovastatin affected the expression of genes associated with DNA replication, Rho/PLC signaling, glycolysis, and cholesterol biosynthesis pathways, suggesting that statins have pleiotropic effects on tumor cells. The above results suggest that repurposing statin drugs for ovarian cancer may provide a promising strategy to prevent and manage this devastating disease.

Moreover, we have started the bisphosphonate (Alendronate) study, and our preliminary results are summarized below.

To determine whether the inhibition of the downstream of mevalonate pathway prevents tumor development, the oviduct glycoprotein 1 (OVGP1) transgenic mouse model with over expressed by the promoter was used in this study (mogp-Tag transgenic mice). Mogp-TAG mice spontaneously develop STICs and ovarian/tubal carcinoma at a relatively young age (6 weeks of age). The transgenic mouse model shows that the tumor development would be invasive epithelial ovarian carcinoma from normal tubal epithelium.

Alendronate suppresses growth of ovarian tumor in OVGp1 mouse

The mice were treated with Alendronate (5mg/kg) or control vehicle beginning at 3 weeks of age. The animals were euthanized at 8 weeks to evaluate tumor burden. We found that the Alendronate significantly reduced the total tumor size in the female reproductive tract (Fig. 1A). The tumor weight was measured in 6 to 12 weeks of age which shows statistical difference in 12 weeks of age (Fig. 1B). In addition, there was no statistical difference in the body weight transition at the beginning of experiments.

To determine whether the Alendronate reduce the formation of tumor, we compared histopathology of fallopian tubes between vehicle-control and Alendronate-treated mice. The immunochemistry images shows the staining of H&E, LAMC-1, and Ki-67 positive cells on tissue sections from fallopian tubes of OVGp1 mice. Fallopian tube sections of vehicle-control mice contained either morphologic features of STICs or tubal carcinoma (top, Fig. 1C). In contrast, we found normal-appearing morphology in tubal epithelial cells of Alendronate-treated mice (bottom, Fig. 1C). The quantitative results show that STICs-associated marker, Laminin C1, and proliferative activity marker, Ki-67, were significantly decreased in Alendronate-treated mice compared with that of the control mice ($p<0.01$) (Fig. 1D).

Immune response in leukocytes and T cell markers

The study demonstrated that Alendronate inhibits the phenotype of tumor development. Next, we determined whether the inhibition of carcinogenesis was via suppressing global immune response to reduce the chronic

environment of ovarian cancer. The immunochemistry of CD45 staining, all leukocytes, shows no difference between Alendronate-treated and vehicle-control in either fallopian tube or ovary (Fig. 2A). In addition to determine if the global immune response decreased in OVGP1 mice with Alendronate treatment, we characterized T lymphocyte populations from spleen. CD3e⁺ exhibited slightly decreased in Alendronate-treated compared with vehicle-control (34.98% vs. 30.83%), but not for the populations of T helper cells (CD4⁺) and cytotoxic T cells (CD8⁺) (Fig. 2B).

Fig. 1

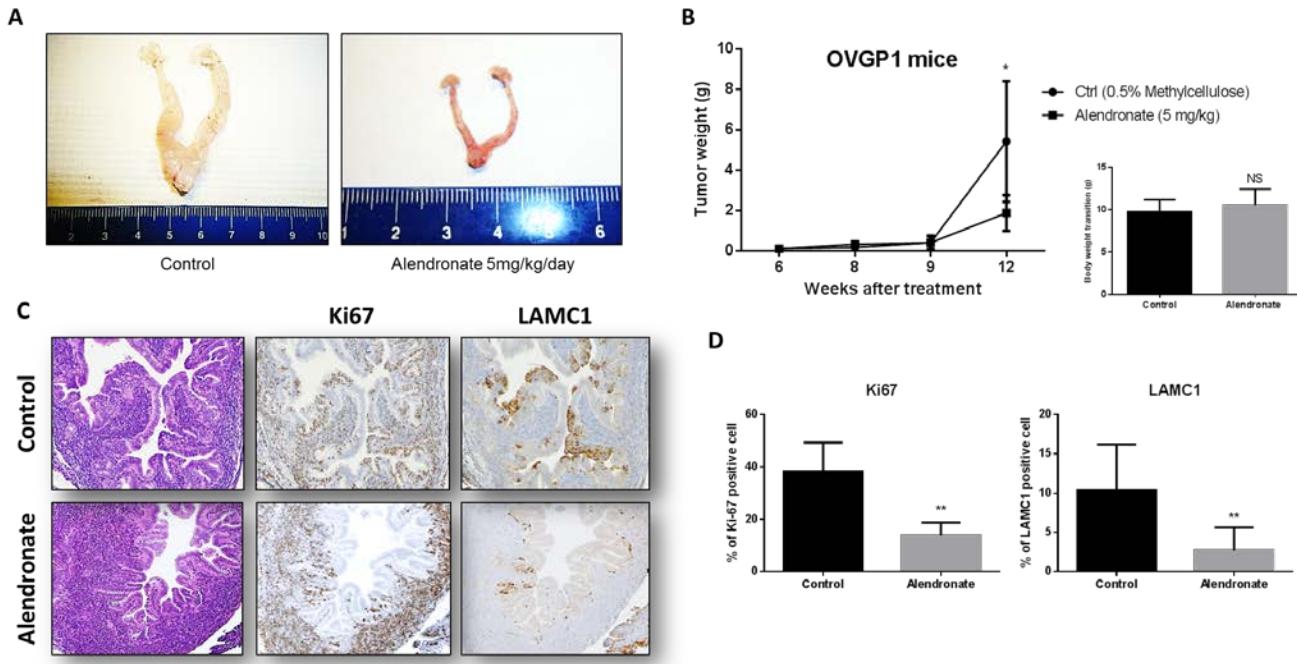


Fig. 1. Alendronate suppresses growth of ovarian tumor in OVGP1 mouse. (A) Alendronate was treated into OVGP1 mice via oral gavage (5mg/kg/day). (B) The weight of GYN organs was measured after harvested ovaries, fallopian tubes and uterus in different age weeks. (C) Representative images of H&E, LAMC-1, and Ki-67 staining on tissue sections from fallopian tubes of OVGP1 mice. (D) Summary of LAMC-1 and Ki-67 staining results. Bar charts depict the percent of LAMC-1-positive or Ki-67-positive epithelial cells among total fallopian tube epithelial cells per section. In each experimental group, data were collected from 5 representative sections from each mouse; Results are expressed as mean \pm SEM. * p <0.05, ** p < 0.01, two-tailed Mann-Whitney U test.

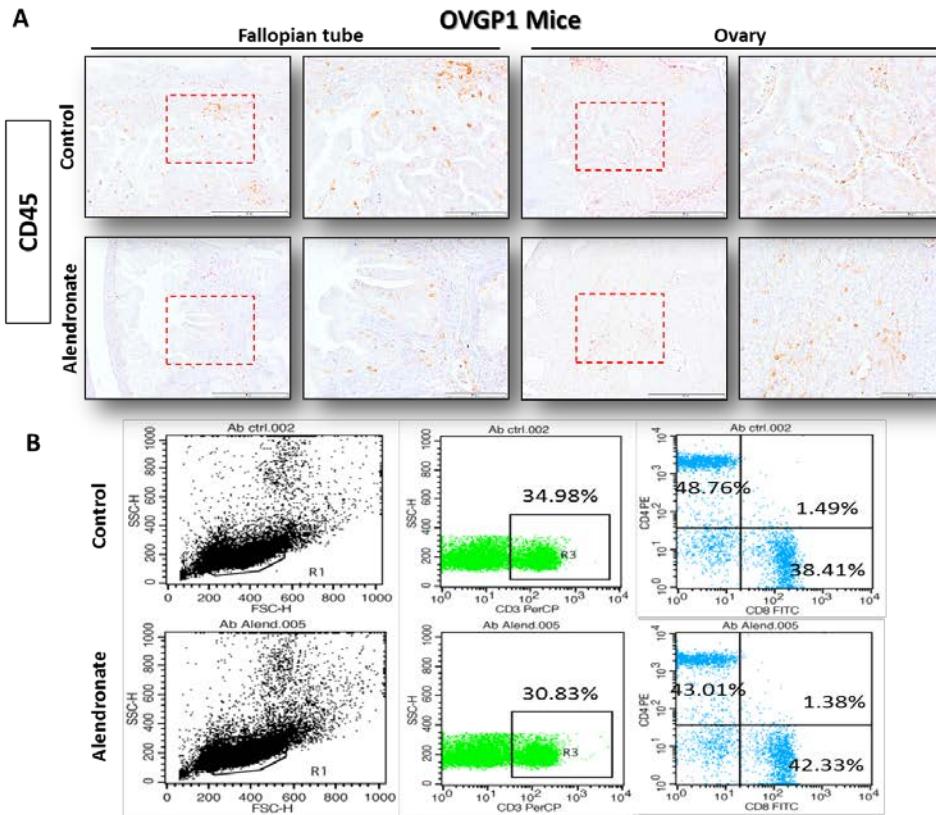


Fig. 2 Immune response in monocytes and T cell markers

(A) Five high power (400X) fields were screened per tumor in fallopian tubes and ovaries, respectively. In total, 10 and 20 high power (400X) fields were included for each experimental group of 5 mice. (B) The effect of Alendronate on T lymphocytes. Cells were harvested from spleen of OVGP1 mice treated with Alendronate (5mg/kg/day) for 4 weeks and then analyzed by flow cytometry after staining with PerCP conjugated CD3e antibody, PE conjugated CD4 antibody and FITC conjugated CD8 antibody.

Task 3c. Data analysis and preparation for publication (24-60 months).

Progress: Our next publication target will focus on reporting that bisphosphonate can reduce the STIC and HGSC formation in the OVGP1-statin mouse model.

Section III. Problem Areas for Project 4

There are no major problem areas noted for Project 4 as alternative approaches are proposed to address the same questions as originally proposed.

Section IV. Future Work in Project 4

Our main objective in the remaining years is to focus on the most exciting finding in Project 4- i.e., the chemoprevention of STIC by statin drugs. As we have published the first paper on chemoprevention, our momentum remains to continue publishing the 2nd paper on bisphosphonate. Specifically, in the next coming half year, we will determine the effect of Alendronate on expression of apoptosis markers and tumor associate macrophages. We will next test whether Alendronate treatment affected on reducing the level of tumor-associated macrophages (TAMs) in OVGP1 mice and in mouse cancer cell lines (ID8 and MOSE).

Reproductive organs and spleen will be harvested after Alendronate-treated for 8 weeks (5mg/kg/day); cells will be incubated with 50 umol/L Alendronate or vehicle control for 0, 24, or 72 hours, and the expression of protein levels of apoptosis and TAM markers will be determined by Western blot analysis in both *in vivo* and *in vitro* experiments. Our expected results are to detect that the protein levels of CD68 reduce in ovary, fallopian tubes, and uterus in the Alendronate-treated mouse compared with vehicle-control. Next, we will design experiments to determine whether Alendronate has a similar effect with statin which is on the upstream of mevalonate pathway, we plan to test the protein expressions of mouse cancer cell lines. Activity of apoptosis based on Cleaved-caspase 3 expression will be detected as early as 24 hours in ID8 cell line after statin exposure; the Cleaved-caspase 3 expression indicates the similar elevated expression after Alendronate treatment, although the overall expression would be lower statin exposure. Histopathology of fallopian tube sections from an Alendronate-treated mouse exhibits reduced numbers of IL-6 and CD68 positive cells. We will also quantify the positive cells of macrophage markers in tissue sections and expect to find that either in IL-6 or

CD68 positive cells in Alendronate-treated mice will be significantly reduced compared with that of the vehicle control group.

Project 5: Determine the molecular and epidemiologic profile of putative precursor lesions in the fallopian tubes and ovaries from women at high-risk for ovarian cancer. In addition, Project 5 will determine if these biomarkers and associated precursor lesions are modifiable by oral contraceptives (OCPs) or anti-inflammatory agents, as OCPs in particular are known to prevent ovarian cancer and impact survival.

Research Site: Johns Hopkins University

Principal Investigator: Kala Visvanathan, MD, MHS

Collaborating Sites: University of Toronto (Steven Narod, MD and Patricia Shaw, MD: Co-investigators), Memorial Sloan Kettering Cancer Center (Douglas Levine, MD and Robert Soslow, MD: Co-investigator), Yale University (Vinita Parkash, MD and Ellen Matloff, MSc, MS, CGC: Co-Investigators)

Section II. Progress to Date:

We continue to work intensively on completing all aspects of project 5 and in the process have come to realize we will need to make further amendments to our original approach based on our initial findings. Despite these changes we are confident that the results of our study will make a significant contribution to our current understanding of the role of STICs in ovarian cancer and help inform the evaluation of future preventive strategies.

1. Pilot Study to evaluate the prevalence of STICs:

This aim was to evaluate the prevalence of STICs by conducting **extensive** sampling of each tumor block in the fallopian tube and ovaries in 114 cases. Sequential eligible cases were identified from Johns Hopkins (37), MSKCC (33), and Yale (44). Toronto was not involved as there pathology department would not allow them to flip the blocks and sample from both ends.

Immunohistochemical staining for p53, Ki67 and Laminin C has been completed on 37 cases (2,880 slides). In addition, H&E stains were done for all 114 cases. Due to time, effort and cost constraints we did instigate the two-tier process mentioned in our last report.

The remaining 77 sequential cases were stained first with Laminin C and H& E slides. Cases that had a STIC on H & E review or were Laminin positive underwent p53 and Ki67 staining and were sent back to the two separate reviewers. Pathology reviews and results for all cases have been entered into a database.

Adjudication was required on a total of 27 cases in which there was discrepancy of final diagnosis. Of the 77 cases stained for Laminin C, there were 6 positive cases that as per protocol we have now stained for p53 and Ki67 staining. We are now at the final stages of adjudication. All pathology reports and minimal clinical data have been uploaded. We plan to start data analysis shortly and then put together and submit a manuscript

2. Pathological and Epidemiological data from the larger prospective/retrospective Cohort:

For this aim eligible participants were identified from 2 prospective cohorts of high-risk women, Hopkins and Toronto and from MSKCC clinical database. We have compiled, cleaned and reviewed epidemiological and clinical data on 400 individuals as well as all pathology reports. We have now analyzed the data and are finalizing our tables. In addition all 19 cases of STIC/Invasive Carcinoma are undergoing central review that includes Ki67 and P53 for STICs.

We had 339 patients who underwent RRSO and had no STICs/Cancer. Sixteen had STIC, 3 had cancer, 9 had p53 signatures, and 34 had inclusion cysts. The prevalence of STICs alone was 4% which is far less then originally reported by pathology case series of women with RRSO.

We report on two interesting observations. The women who developed STIC/cancer were all over 40 and on average were significantly older than those who did not develop any. This may explain why the prevalence of STICs is so low in women undergoing RRSO. Secondly we observed that statin users were significantly less likely to have STICs/invasive carcinoma on presentation that is consistent to what we have observed clinically.

This will be the second largest study published and the first outside a clinical study which can often be less reflective of the general population.

3. Molecular and Epidemiological Characterization of STICs:

As indicated in our last progress report we continue to focus efforts on molecular characterization of STICs as it will help target evaluation of potential preventive strategies. We have made progress towards a case control study (without expanding eligibility criteria). In order to enrich STIC cases to 40+ each site conducted a search for additional STIC only cases with adequate tissue from their pathology database along with a control (matched on date of surgical procedure, site and age). To date, Hopkins identified 4 STIC cases, MSKCC 12 STIC cases and Toronto 12 STIC cases. We are in process of reaching out to other potential external sites to identify additional STIC cases and will amend our research plan to include these sites once we have a firm commitments. At present we have approximately 38 total possible STIC cases and 30 controls. The controls are from the same site. We are establishing IRB protocols and MTAs with each site. Our goal is 50 STICs.

Pending the slide availability we will prioritize our markers. We plan to acquire our target goal by the end of the year. We will then compare molecular markers in the STICs to the adjacent normal as well as to fimbrial end of the fallopian tube of women without STICs. We feel strongly that this will be a unique contribution to the field as it will provide important information about the etiology of STICs and will also help guide preventive strategies.

Specific Tasks

Task 1. Obtain approval for the addition of questions for prospective collection from all site IRBs (Aim 1) and also for the transfer of existing epidemiological and clinical data to JHH de-identified for the retrospective study (Aim 1 and 2). Obtain approval from USAMRAA for human subject's research for entire protocol (Months 1-6).

Progress: This task was completed. We continue to maintain current IRB approvals at each institutional site for subsequent studies.

Task 2. Identification of study population for retrospective study based on eligibility criteria from all sites (Months 1-6).

Progress: This task was completed. All epidemiological data and pathology reports on 400 cases have been identified, collected, entered, cleaned and analysis and manuscript preparation is in process.

Task 3. Comprehensive pathology review of samples. A comprehensive master dataset that includes epidemiological and pathological data from approximately 550 women to be used for cross-sectional and case control study designs will be created (Months 6-18).

Progress: Task completed. We compiled an epidemiological and pathological database of 400 high-risk women due to the low prevalence of STICs we decided not add further to this database.

Task 4. Analyses for Aims 1a and b will be completed to determine the prevalence, location and frequency of the specific lesions in the FTEs (STICs, STILs, and p53 signatures) and OSE, as well as CICs in the ovaries,

overall and by BRCA mutation status. Correlations between each of the different types of lesions will be determined. Manuscript preparation Aims 1a and b (Months 6-18).

Progress: We will now look at prevalence from two perspectives. The first is a detailed pathological and molecular evaluation of 114 cases for STICs sampling from both the top and bottom of each block. Secondly we will report on the prevalence of 400 high-risk women who underwent prophylactic oophorectomy. The first manuscript is in preparation the second will follow soon once the adjudication is complete.

Task 5. Analyses for Aim 1c the association between exposures (both risk and protective factors for ovarian cancer) and each type of prevalent lesion will be examined through cross-sectional studies. Manuscript preparation for Aim 1c (Months 12-24).

Progress: This manuscript is in process for the 400 cases identified (see Task 4).

Task 6. As described above, merging of epidemiological and pathological data for aims 1 and 2 will be performed and a comprehensive master dataset to be used from which matched case-control studies will be identified for Aim 2 (Months 6-18).

Progress: This task has been completed. However given the low prevalence of STICs among 400 cases we are reaching out to other sites for STICs.

Task 7. Case-control sets for molecular analyses of the FTE and ovary samples from Aim 1 will be used to assess a panel of markers. Cases will be defined by the lesion/region of interest (i.e. STICs, STILs, p53 signatures, CICs, and/or morphological changes in OSE). Controls will vary depending on the analysis: marker expression within a specific lesion will be compared to (1) adjacent normal tissue from the same case and (2) normal tissue from women with no identifiable lesion. For the latter comparison, 2 controls will be matched to each case from the same research site, within +/- 2 years of age at the time of surgery. To conserve this valuable tissue, and maximize efficiency, the same controls will be reused for subsequent case-control analyses where possible (Months 18-24).

Progress: We have made progress in expanding the current study to include all STICs irrespective of high-risk status. We are in process of contacting additional external sites to discuss potential collaboration for STIC cases (i.e. Cedars-Sinai, UCLA, Northwestern, Rush University, etc.). We have identified 38 potential cases in our discussions. We are aiming to locate 40-50 STIC cases and matched controls as described above. So far we have contacted 20 external institutions for 38 STICs.

Task 8. Molecular analyses to determine molecular profile of each lesion type will be performed on case control sets. The resulting laboratory results will be merged with existing epidemiological data and analyses will be performed (Months 24-48).

Progress: Once the case-control sets are finalized which we expect by end of year we will embark on molecular analysis in the first 6 months of 2016.

Task 9. Statistical analyses of case-control sets will be completed for each lesion type and panel of markers. Multiple manuscripts will be generated from Aim 2 based on the different lesions and also markers. These will be prepared and submitted (Months 24-48).

Progress: This will be done in 2016.

Task 10. High-risk women considering BSO in the next 2 years, and meeting the same eligibility criteria used in Aims 1 and 2, will be prospectively enrolled at each site. Information on NSAIDs, OCP and Vitamin E use as well as other ovarian cancer risk/protective factors will be collected through questionnaires completed within 2 years of surgery (N ~ 300-400) (Months 6-48).

Progress: Status remains same as last report. Given the low prevalence of STICs we will not proceed but have directed our focus this period of identifying unique case-control set from various institutions.

Task 11. Ongoing comprehensive pathological review and merging of epidemiological and pathological data will occur (Months 6-50).

Progress: This has been done for the master database and pilot.

Task 12. Complete merging and data management of Master data set (Months 48–50).

Progress: Completed and data is in review and being analyzed.

Task 13. Analysis of prospective data with respect to NSAIDs and OCP use will be completed: a) Associations between OCP/NSAID/Vitamin E use and prevalence of lesions will be evaluated, overall and stratified by BRCA mutation status as in Aim 1, and b) associations between use of these substances and molecular markers identified in Aim 2. Manuscripts for Aim 3 will be prepared and submitted. This aim will be informed from data generated in Aims 1 and 2 (Months 48-60).

Progress: We have completed our initial data review and analysis from both prospective and retrospective data regarding these medications. Given the low prevalence of STICs and low prevalence of this exposure in this population we will not undertake a prospective study.

Section III. Problem Areas of Project 5

There were no new challenges since last project report.

Section IV. Future Work in Project 5

In the next 6 months we will submit 2 manuscripts for the epi study and the pilot study, complete collection of STIC case control sets and begin molecular characterization of STICs.

Administration (Admin) Core, Biostatistics/Bioinformatics/Epidemiology (BBE) core, and Pathology (Path) Core

As described in the previous progress reports, the integration of the three cores has been integrated to each other; therefore, we combine their progress in this section to avoid reiteration. Because the tasks related to the three cores are relatively generic and are applicable for the entire research period, so we will rather report the specific progress related to the cores.

Section II. Progress to Date:

In the last 6 months, the Administration Core led by Drs. Kurman and Shih continues providing all the administrative support to all 5 research projects. Communications among project leaders have become a routine through several venues as described in previous reports. As described in the last progress report, the Administration Core has help organized the face-to-face meeting of key personnel within this consortium through the venue of the annual meeting of the United States and Canadian Academy of Pathologists (USCAP) at Boston Convention Center in March 2015 and in the AACR Ovarian Cancer Congress at Orlando in October 2015. The participants discussed the progress in individual projects, potential issues related to this consortium and realign all the projects into the goal of this program to further elucidate the pathogenesis of ovarian cancer precursors.

The BBE core provides excellent support to the current study design and assists statistical analysis for all the data generated in the past 6 months. The BBE core is currently working with investigators to prepare for manuscripts to be submitted to journal publications in the future.

The Path Core led by Drs. Visvanathan, Soslow, Kurman and Shih continues to serve as our central collection resource for the various projects. Highlights of Path Core progress this past period include:

Path Core Activities since September 2015

- 1- Supported ongoing efforts for sites with slide shipping, labeling, and data entry.
- 2- Continued to enter slides into the database upon receipt from sites. To date, > 38,000 slides have been entered and cataloged between Toronto, Yale, MSKCC and Hopkins (see table below). There are remaining slides to enter for Hopkins and we anticipate this will be completed in the next few weeks.
- 3- Coordinated a systematic slide cabinet storage check to ensure all slides properly integrated and organized.
- 4- Continued to trouble shoot site issues regarding use of the repository database.
- 5- Provided continued oversight of uploading of path-reports by all study sites. A total of 560 have been successfully de-identified and uploaded (see table below).
- 6- Completed all slide staining for Project 5 pilot for each study site.
- 7- Coordinated and finalized the 2 re-reviews of each Project 5 pilot case as well as the adjudication process. A total of 7,680 slides were re-reviewed and 27 cases required adjudication and 6 cases required further staining and a third confirmation review. All slide re-review and adjudication scoring was entered into a separate re-review database.
- 8- Obtained JH local IRB Continuing Approval for the Path Core/Ovarian Biorepository on October 14, 2015.

Total Cases in Repository: Breakdown by Institution as of 10/19/2015

	JHM	MSKCC	Yale	Toronto	Total
Ovarian Cancer Cases	29	15	10	25	79
Prophylactic Project 5 Cases	108	300	47	199	654
Total	137	315	57	224	733

Total Blocks and Slides in Repository: Breakdown by Institution as of 10/19/2015

	JHM		MSKCC		Yale		Toronto		Total	
	Block	Slide	Block	Slide	Block	Slide	Block	Slide	Block	Slide
Ovarian Cancer Cases	36	1931	17	828	10	515	40	2346	103	5620

Prophylactic
Project 5

Cases	0	0	98	4310	131	4409	354	24086	583	32805
Total	36	1931	115	5138	141	4924	394	26432	686	38425

**Total de-identified Pathology Reports in Repository: Breakdown by Institution as of
10/19/2015**

	JHM	MSKCC	Yale	Toronto	Total
Ovarian Cancer Cases	29	15	0	25	69
Prophylactic Project 5 Cases	97	152	44	198	491
Total	126	167	44	223	560

Section III. Problem Areas for the Cores

As discussed in the last report, we did not have sufficient fund for the 2015 Ovarian Cancer Consortium meeting. As a result, the Admin Core has arranged a group meeting taking advantage of venue of annual meeting like USCAP so the key investigators could meet and discuss. It turned out that such arrangement of meeting was as effective as having an official meeting. Besides, there were frequent discussions among all project leaders and core directors. Otherwise, we do not expect major problems for the three cores.

Section IV. Future Works in Cores

The future works for the three cores correspond to what have been proposed in this consortium study. Briefly, the Admin Core will continue overseeing and manage performance of the Path and BBE Cores and the Research Sites. Specifically, the Admin Core will promote the integration and coordination of research activities, ensure new IRB protocols and additional biosafety regulations are in place, coordinate intellectual and material property, organize regular meetings of the investigators including the Annual Ovarian Cancer Symposium, monitor project performance, prepare annual progress reports, organize and make travel arrangements for investigators and ensure dissemination of data and publication of major findings as well as maintaining the ovarian cancer web site. The Admin Core will plan the next Ovarian Cancer Symposium and this task will be the priority for this Core. The Path Core will be responsible for tissue repository and the BBE core will ensure that data are consistently collected and entered into the Consortium database. As described in the task, the Path Core will collect tissue and analyze it histologically according to standardized practices and the algorithm that we developed for this program, perform tissue-based assays, and annotate this material in a centralized fashion while assuring quality. The Path Core will also oversee functions related to storage and transportation of tissue samples in order to supply researchers with pathologically characterized material. Both Path and Admin Cores will work together to coordinate the shipment of slides among Research Sites and the Pathology Core. The BBE Core will provide biostatistics, bioinformatics, and epidemiology consultation and support to all members of the Consortium. Core members will assist in the design, collection, storage, visualization, analysis, quantitative modeling, and interpretation of the data arising in the course of Consortium activities. Centralizing biostatistical and bioinformatics support within the Core will ensure that the necessary expertise will be available for all projects, facilitating the coordination and integration of data throughout the Consortium in a consistent fashion. Dr. Cope in BBE core will analyze the profiling data collected from Project 2.

Section V- Administrative Comments (Optional)

Section VI- Meetings

We will consider having the next DoD Consortium PI meeting in conjunction with the USCAP annual meeting at Seattle in 2016.